[redacted]

1005075403

CTR GRANTS 1974

Harry L. Ioachim, M.D.
Department of Pathology
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100 East 77th Street
New York, New York 10021

The Immune Response in Lung Carcinoma.

The present project is based on the assumption that the tumor site is the most favorable place for investigating the immune response to the growth of a tumor, since antigens, antibodies and reactive lymphocytes are more concentrated there than in the general circulation.

Using both pleural effusions and eluates of solid tumors, immunoglobulins were recovered according to procedures previously described. It was found that the fractions isolated from both eluates and effusions consisted almost entirely of IgG with small amounts of IgA and IgM and very little other serum protein contaminants. The immunoglobulins isolated from 9 eluates and 16 effusions of adeno and squamous cell carcinomas of the lung were tested in indirect immunofluorescence against tissue cultures and suspensions of lung carcinomas. They exhibited bright positive immunofluorescence in almost all cases and showed no reaction with tissues of normal adult and fetal lung or with cells of nonpulmonary carcinomas. Six effusions of nonpulmonary tumors used as controls showed no such reactivity.

Preliminary assays were recently conducted with bronchial washings of patients with and without lung cancer, from which immunoglobulins were isolated and assayed in indirect immunofluorescence. The results of the first washings tested, were similar to those obtained with eluates and effusions. More cases will be screened in order to assess the potential value of this technique.

Progress was also made in the preparation of immunoadsorbents to be used in the recovery of lung tumor antigens needed for heterologous immunization. Immunoglobulins isolated from effusions were absorbed with an insoluble normal human serum-normal lung polymer, then polymerized with ethylchloroformate to be used as an immunoadsorbent for the recovery of tumor-associated antigens from the extracts of human lung carcinomas. This material will be used to immunize rabbits in order to produce a high titer heterologous antiserum against lung tumor-associated antigens.

Activation Date: January 1, 1978

Current Grant Level: $41,000.

Jay A. Levy, M.D.
Cancer Research Institute
School of Medicine
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San Francisco, California 94143

Possible Genetic Determinants of Chemical Carcinogenesis.

The objective of this project is to develop a model system in vitro for studying carcinomas. Possible interplay of endogenous viruses and chemicals in cocarcinogenesis will be investigated here.

Continued studies on the genetic basis for viral and chemical carcinogenesis will be conducted by breeding experiments using NZB, 129/J and SWR mice. NZB mice spontaneously produce significant titers of xenotropic virus. 129 and SWR produce very little. The possible interplay of X-tropic virus production and chemical induction of transformation will be examined by using F_1, F_2 and backcross generations.

(NZBx129)F_1 produce significant titers of infectious xenotropic virus whereas 129 mice alone do not. Testing of the NZBxSWR and the backcross generations for virus production is in progress. Concomitantly, these mice will be tested for susceptibility to tumor induction by 3-mercaptoethanol.

Activation Date: April 1, 1977 - June 30, 1978

Current Grant Level: $65,474.
Supplement: $16,368.50

R. Russell Martin, M.D.
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Baylor College of Medicine
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Induction of Aryl Hydrocarbon Hydroxylase in Lymphocytes and Pulmonary Macrophages.

The objectives of this research are to study induction of aryl hydrocarbon hydroxylase (AHH) in mitogen-stimulated peripheral blood lymphocytes and in pulmonary alveolar macrophages (PAMs) from healthy volunteers, including both nonsmokers and cigarette smokers. Induction of AHH in both lymphocytes and PAMs after in vitro incubation with water soluble extracts of tobacco smoke will also be employed with short-term cultures. Finally, studies will be performed to determine the ability of PAMs from nonsmokers and smokers to mediate macrophage-dependent lymphocyte responses in vitro. These investigations will examine the possibility of individual variation in inducibility of the broad function microsomal-enzyme system AHH.

Activation Date: January 1, 1978

Current Grant Level: $27,750.

Microbiological Associates
5221 River Road
Bethesda, Maryland 20016

Human Aryl Hydrocarbon Hydroxylase Studies.

The object of this study is to achieve a reproducible AHH assay system applicable to human subjects. The use of this assay may reveal a relationship between levels of AHH and carcinoma of the lung, as well as other cancers that have been assumed to be related to environmental carcinogens.

This past year, four methodologic changes have resulted in an assay for AHH activity in cultured human lymphocytes that is much more reproducible than any assay method previously available. These changes are: (1) use of human AB serum in the culture medium; (2) use of NADH-dependent cytochrome c reductase activity as the measure of total microsomal content in mitogen-activated lymphocytes; (3) correction for the percent of T lymphocyte cells in the initial assay culture; (4) use of frozen (-120° C) stored lymphocytes as source material of AHH determinations. This latter point is particularly important because it may offer a practicable method for analyzing the role of AHH activity in the cancer susceptibility in man. With proper confirmation in other laboratories these methodologic changes may permit an inquiry into genetic regulation of AHH levels and the role of these hydrocarbon metabolizing enzymes in cancer susceptibility in man.

In a related program, twin studies and family studies will be carried out in order to determine whether AHH levels are under some form of genetic control. In collaboration with Drs. H. Lynch and H. Guirgis (CU), lymphocyte samples from specific family members will be collected, stored at -120° C and assayed after accumulation of samples from families congenitally either susceptible or resistant to certain forms of cancer. The "Cancer Family Syndrome" population is characterized by an increased frequency of adenocarcinomas of all varieties, but predominantly adenocarcinomas of the endometrium and colon, increased frequency of multiple primary malignant neoplasms (20% greater), earlier age of onset of cancer as compared to its occurrence in the general population, and finally, autosomal dominant mode of inheritance. By analogy with in vivo model systems using inbred strains of mice, it is possible that in at least some of the cancer-prone families the rate-limiting factor controlling susceptibility may be their inherent capacity to metabolize chemical carcinogens.

Activation Date: January 1, 1978

Current Contract Level: $148,000.

Franz Oesch, Ph.D.
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Transformation of Eucaryotic Cells by Polycyclic Hydrocarbons Occurring in Cigarette Smoke: Discrimination between the Importance of Various Enzymes.

At present, these investigators are studying the role of the mammalian enzymes epoxide hydratase and dihydrodiol dehydrogenase in the inactivation of mutagenic metabolites produced from polycyclic hydrocarbons occurring in cigarette smoke using the bacterial test system of Ames. Addition of pure epoxide hydratase reduces the mutagenicity of benzo(a)pyrene when activated by liver microsomes from control mice, but increases the mutagenicity when microsomes from methylcholanthrene-treated mice are used for activation. Epoxide hydratase has no effect upon the activation of the 7,8- and the 9,10-dihydrodiols of benzo(a)pyrene. Preliminary results show that, in contrast, pure dihydrodiol dehydrogenase has no effect upon the mutagenicity of benzo(a)pyrene in the presence of control microsomes, but reduces the mutagenicity in the presence of microsomes from methylcholanthrene-treated mice. Control microsomes activate benzo(a)pyrene mainly to simple epoxides which can be inactivated by epoxide hydratase whilst microsomes from methylcholanthrene-treated animals activate benzo(a)pyrene mainly to dihydrodiol epoxides which are not inactivated by epoxide hydratase. However, the formation of these dihydrodiol epoxides can be reduced by dihydrodiol dehydrogenase. Thus both enzymes complement each other in inactivating mutagenic benzo(a)pyrene metabolites.

These encouraging results are leading the researchers to investigate the role of these enzymes by using the transformation of eucaryotic cells as an indication of carcinogenic activity. To perform these experiments they must (1) establish various in vitro cell transformation systems, (2) develop sensitive assays for the determination of the relevant enzyme activities (monooxygenase, epoxide hydratase, dioldehydrogenase and glutathione S-transferase) in the cultured cells, and (3) study the effect of addition of pure epoxide hydratase and dihydrodiol dehydrogenase to the cultures in the presence or absence of liver homogenate upon the cell transformation caused by polycyclic hydrocarbons.

In parallel, the investigators intend to study the interindividual variation in dihydrodiol dehydrogenase and epoxide hydratase activities in man using skin fibroblast and/or lymphocyte cultures. Activities will also be compared in cells from healthy individuals and from lung carcinoma patients.

Activation Date: January 1, 1978

Current Grant Level: $45,000.

Marilyn A. Rasco, Ph.D.
Assistant Biologist & Assistant
Professor of Biology
The University of Texas System Cancer Center
M.D. Anderson Hospital & Tumor Institute
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Hydrocarbon Metabolizing Enzymes and Lung Cancer.

Aryl hydrocarbon hydroxylase (AHH) is an inducible enzyme which is critical in converting polycyclic aromatic hydrocarbons to their carcinogenic forms. In experimental animals, high inducibility of the enzyme in the lung correlated with increased incidence of lung tumors in response to intratracheal instillation of aromatic hydrocarbon carcinogens. In humans, AHH has been measured in cultured lymphocytes, pulmonary alveolar macrophages, surgically-excised lung tissue and placenta. Initial studies by Kellermann et al. suggested a relationship between high inducibility of AHH in cultured lymphocytes and the occurrence of bronchogenic carcinoma. Other laboratories have had difficulty repeating this work, primarily due to variability in the lymphocyte culture system.

These investigators have attempted to define the sources of variation in lymphocyte AHH activities. Measurements on over 500 cancer patients and 1,000 normal donors indicate that bronchogenic carcinoma patients and oropharyngeal squamous cell carcinoma patients have higher AHH inducibility than the normal population. A case-control study on lymphocytes from 29 lung cancer patients and their spouses confirmed the higher AHH inducibility in the patients.

The researchers have two major goals for this work in the coming year: (1) to further define sources of variation in the lymphocyte culture system and develop appropriate controls; and (2) to identify the cause-and-effect relationship between high AHH inducibility and the occurrence of certain tumor types.

Activation Date: January 1, 1978

Current Grant Level: $50,142.

Nathan H. Sloane, Ph.D.
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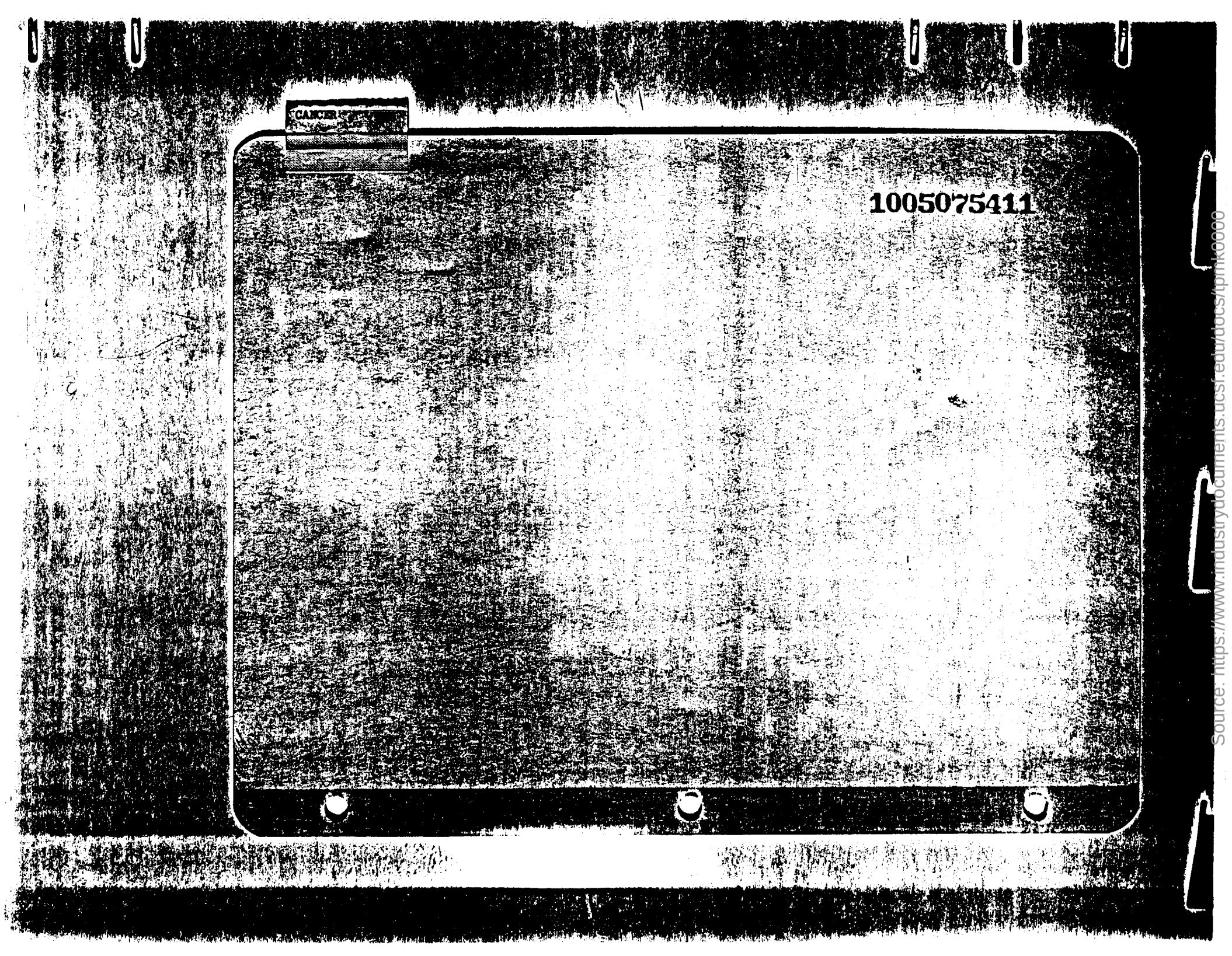
Mammalian Lung Enzyme: 6-Hydroxymethylbenzo(a)pyrene Synthetase -- Biochemical and Biological Studies.

The studies involve the biochemical and biological properties of the carcinogen, 6-hydroxymethylbenzo(a)pyrene. The mammalian lung synthetase is to be isolated and the role of lipid cofactors on the activity of the enzyme will be determined. The role of cytochrome P-450 independent pathways in transformation of tissue culture cells by polynuclear hydrocarbons will also be studied.

Activation Date: January 1, 1978

Current Grant Level: $12,000.



Cancer Studies

1005075412

Joseph C. Arcos, D.Sc.
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Synergistic Effects of Polycyclic Hydrocarbons and Nitrosamines in Pulmonary Carcinogenesis. Potential Repressors of Metabolic Activation of Nitrosamines.

The pulmonary syncarcinogenic effect of 3-methylcholanthrene (MC) and dimethylnitrosamine (DMN) in rats and mice suggests that the lung cancer incidence of smokers is due to synergism between carcinogenic hydrocarbons and nitrosamines present in the smoke, rather than to the hydrocarbons alone. Epidemiological findings indicate that roofing workers, exposed to the inhalation of enormously higher levels of benzo(a)pyrene than are even heavy smokers, have a lung cancer incidence not significantly higher than the general population.

The enzymological study of the mechanism of hydrocarbon-nitrosamine synergism is being continued by determining arylhydrocarbon hydroxylase (AHH), DMN-demethylase, epoxide hydrase and glutathione-S-epoxide transferase activities following acute and chronic administration of MC and DMN. There is firm evidence that pulmonary epoxide hydrase activity is depressed by DMN. The effect on epoxide hydrase appears to be dose-dependent, in accord with the existence of two DMN-demethylase isoenzymes. There is generally substantial difference in all these enzyme responses in AHH-inducible and -repressible mouse strains.

Activation Date: July 1, 1976

Current Grant Level: $67,279.

William H. Fishman, Ph.D.
Tufts Cancer Research Center
Tufts University
136 Harrison Avenue
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Cancer Phenotype Profile which May Presage Bronchogenic Cancer.

The basic premise of this multidisciplinary three-year program is that a fundamental manifestation of neoplasia is the activation of embryonic, placental and fetal genes. The question of prime importance which arises from this premise is, "Do carcinoembryonic proteins appear in the bronchial epithelium of individuals destined to develop bronchogenic cancer?"

Standard conditions have been defined for securing satisfactory tracheobronchial cell suspensions for cytological, cytochemical, biochemical and radioimmunochemical studies. These include peroxidase-labeled antibody technique for Regan isoenzyme, immunoelectrophoretic tests for trophoblast alkaline phosphatase isoenzymes, and radioimmunoassay for Regan isoenzyme and human chorionic gonadotrophin.

An effort will be made to include radioimmunoassay measurements of α-fetoprotein and of carcinoembryonic antigen along with Regan isoenzyme and human chorionic gonadotrophin in the investigators' search for products of activation of embryonic genes in bronchial cells. In addition to cells collected at autopsy, bronchial washings and sputum, specimens will be examined for expression of embryonic genes in their cell populations.

Activation Date: May 1, 1976

Current Grant Level: $51,368.

William F. Benedict, M.D.
Division of Hematology-Oncology and Medical Genetics
Childrens' Hospital of Los Angeles
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Malignant Transformation, Mutagenesis and Fibrinolysin Production of Cigarette Smoke Condensate Fractions.

The researchers plan to investigate various fractions of cigarette smoke condensate for their ability to be promoters as well as initiators of the carcinogenic process using the C3H/10T1/2CL8 mouse cell line developed in the laboratory of Dr. Charles Heidelberger for transformation studies. They shall also continue to investigate whether the same fractions which transform the mouse cells are similar to those which are mutagenic in the Salmonella histidine mutagenesis system developed by Dr. Bruce Ames. Finally, they shall study chromosomal aberrations produced by these same cigarette smoke condensate fractions in the hamster cell line A(T_1)C1-3 which was developed in their laboratory.

Activation Date: June 1, 1976

Current Grant Level: $47,714.

Arthur Furst, Ph.D.
Director
Institute of Chemical Biology
University of San Francisco
San Francisco, California 94117

Induction of Squamous Cell Lung Carcinoma in the Mouse.

The objective of this contract is to determine whether squamous-cell lung carcinoma analogous to the human disease can be induced in the laboratory mouse by methods similar to those reported by Saffiotti to have been successful with the hamster, namely, the intratracheal instillation of measured doses of known carcinogenic agents along with ferric oxide.

Mice have the advantage of availability in many inbred strains with contrasting degrees of cancer susceptibility as shown by empirical studies and defined in some part by observed differences in aryl hydrocarbon hydroxylase inducibility and in endogenous C type viral genome and oncogene expression. Their disadvantage is the small lung dimensions which impose technical mechanical problems.

Successful adaptation of the intratracheal instillation techniques is now being followed by systematic studies of tolerance levels for benzo-a-pyrene and methylcholanthrene, clearance rates of these hydrocarbons and of iron oxide, pathological changes in the lung as related to time of contact and dosage level of these substances, yield and nature of lung neoplasms observed, and the influence of various vehicles for the hydrocarbon administration.

The study has been extended to instillation of other particulate materials, especially chrysotite asbestos, with observation of distribution within the lung, rates of clearance and pathological effects with time.

These investigations are intended to prepare the way for long-term chronic studies of cigarette smoke inhalation in defined mouse systems.

Activation Date: June 1, 1974 - March 31, 1975

Current Contract Level: $75,000.

Murray B. Gardner, M.D.
University of Southern California
School of Medicine
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A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer. Grant #714R1

Our program identifies within its total scope four principal interrelated research areas: Viral Studies, Epidemiologic Studies, Environmental Studies, Chemical-Viral Cocarcinogenesis Studies. We plan to work with several strains and species of animals including feral and laboratory mice and domestic cats and to use chemicals collected from Los Angeles environment and synthetic chemicals of known composition. In tissue culture the use of rodent and human cells that have been infected (productive or nonproductive) with C-type virus from a heterologous host (mouse and cat) will constitute advantageous types of indicator cells upon which to test this chemical-viral interaction. Assay systems include electron microscopy for the presence of virus particles, serologic techniques for detection of C-type viral gs and/or envelope antigen, biochemical studies for specific classes of RNA, COMUL and COCAL testing for covert infectious C-type virus and tests for virus transmissibility in appropriate animal hosts. Using these test systems we plan to compare the effect of environmental chemicals fron one area of L.A. to another and to correlate the laboratory observations with the epidemiologic field observations in the occurrence of specific types of cancer in man (and animal) within the same geographic areas. We also plan to study the effect upon C-type viral activation and cancer induction of other factors such as maternal age, number of gestations, hormones and drugs. We plan to work with the domestic cat for experimental studies upon chemical-viral oncogenesis as well as for natural history studies on the feline RNA tumor virus. We already have indications that human sarcoma cell lines, which have been cloned and propagated in tissue culture, can be successfully transmitted to cat fetuses in utero. This suggests that the cat fetus may be an unusually advantageous route for propagating human tumor cells; it presents an exciting opportunity to look for cat-human hybrid tumor cells or, conceivably, for rescue of a hypothetical defective human sarcoma genoms with the feline leukemia virus envelope.

Current Grant Level: $54,150.

David M. Goldenberg, Sc.D., M.D.
Temple University School of Medicine
Philadelphia, Penna.

Heterotransplantation Studies with Human Lung Cancer. Grant #640R2

The initial objective of this project is the establishment of several serially transplantable human bronchogenic carcinomas in the cheek pouch of the golden hamster. Various methods of conditioning the foreign host will be examined in order to provide the most optimal means for attaining survival and propagation of such xenogeneic neoplasms. Data to date has shown that lung tumors of human origin can be grown at this site in unconditioned adult hamsters, and that these serially transplantable neoplasms have attained highly malignant growth patterns (invasiveness and metastasis) in their animal hosts; two such permanently propagable cell lines are "GW-365" and "GW-426." The cytologic and genetic alterations and/or constancy of various cell types within such heterogeneous cell populations of bronchogenic carcinomas are currently being focused upon with regard to questions of histogenesis and possible host-induced alterations. Current evidence strongly suggests that in vivo hybridizations of human cancer and normal hamster genomes can at times occur, and that this event might have definite implications for the progression of malignancy in man. The possible role of certain host-borne viruses in the process of somatic cell fusion is also being investigated.

Current Grant Level: $33,332.

Harry L. Ioachim, M.D.
Department of Pathology
Lenox Hill Hospital
100 East 77th Street
New York, New York 10021

Studies of Human Lung Carcinomas.

The objective of these studies is to investigate the immune reactions to lung carcinoma. It is expected that in addition to a better understanding of the immune reactions, specific antigens and/or antibodies will be detected that could be used in the diagnosis of these tumors.

The possibility that antigenic components similar to those of epidermis might be released by squamous cell carcinomas of the lungs and that specific antibodies against them might be formed during the growth of these tumors is being investigated. This study uses indirect immunofluorescence and complement fixation tests for parallel investigations, and includes sera of patients with squamous and nonsquamous carcinomas of the lung, with squamous and nonsquamous carcinomas of other organs, and with various inflammatory lesions as well as sera of normal controls.

Extensive screening of sera will be conducted including those of patients with squamous and nonsquamous tumors of the lung, with squamous and nonsquamous tumors of other organs, and with non-neoplastic conditions, as well as sera of normal controls. Increasing the number of cases in each category is very important at this stage in order to fully assess the significance of these autoantibodies. The results of the screenings in complement fixation and indirect immunofluorescence tests will be correlated with a careful analysis of patient's history as well as with the presence of other serum antibodies such as antinuclear antibodies and antiblood group antibodies.

Activation Date: January 1, 1975

Current Grant Level: $44,000.

Richard A. Lerner, M.D.
Scripps Clinic and Research Foundation
476 Prospect Street
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Studies on Persistent Viral Infection.

The objectives of this project are to understand in detail the host virus interrelationship, especially from the immunopathologic point of view, for oncornaviruses. The investigators will continue to characterize in molecular and immunological terms the oncornavirus which is spontaneously shed from the New Zealand thymocytes which are in continuous suspension culture in their laboratory.

They will use the usual methods of molecular biology to characterize the structure of this virus (see above). In addition, an extensive investigation relative to the immunopathological consequences of infection with this virus will be carried out.

The items mentioned in the preceding paragraphs are already supported by The Council for Tobacco Research, and current plans are simply to continue the investigation along the avenues already initiated.

Activation Date: July 1, 1974

Current Grant Level: $55,000.

Richard Lerner, M.D.
Department of Experimental Pathology
Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

Immunological Competence and Chemical Carcinogenesis.

The specific research aim of this study is to elucidate the possible role played by the host immune mechanism during chemical carcinogenesis. Initially, the basic immunological status of several strains of mice was examined. Plaque forming responses to six different antigens were measured in both sexes of each of seven strains of mice. This involved individual assays on over 1500 mice. Based on these data, in conjunction with Drs. Whitmire and Kouri of Microbiological Associates Inc., it was decided to utilize strains C3H, DBA/2 and C57B1/6 for the second phase of the study with 3-methylcholanthrene (3-MC). Goat erythrocytes were selected as the test antigen, and the kinetics of the immune response to goat erythrocytes were measured in each strain.

The cooperative phase of the study with Drs. Whitmire and Kouri at Microbiological Associates Inc., which aims to determine possible impairment of the systemic immune response following a large intratracheal dose of the chemical carcinogen 3-MC, is now beginning. Strains selected for testing represent each major histocompatibility group, high and low tumor susceptibility strains, and aryl hydrocarbon hydroxylase inducible and noninducible strains, each of which has been shown to be immunocompetent by the studies completed in the first phase of this study. At Microbiological, either 500 µg 3-MC suspended in gelatin, gelatin alone or nothing will be administered intratracheally to each mouse. These will then be sent to Scripps where on the sixth day an intravenous immunization with goat erythrocytes or saline will be given. Ten days after that, a booster injection will be given and the antibody forming cells in the spleen of each animal will be determined at various times. Plaque forming cells will be assayed six separate times for each strain and sex, on each of four days after second immunization so that both quantitative suppression and possible changes in kinetics of response will be determined.

This protocol in the future will be adapted to determine the immunological consequences of different chemical carcinogens and tobacco smoke.

Activation Date: July 1, 1974

Current Contract Level: $59,750.

*-This contract was drawn to augment and service the work being done by Microbiological Associates Inc. in Contract 14. (See A-21.)

Jay A. Levy, M.D.
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School of Medicine
University of California
San Francisco, California 94122

Development of a Model System In Vitro for Studying Carcinomas.

Over 80% of human cancers, particularly those of the lung, are carcinomas arising from normal epithelial cells. Nevertheless, most of the in vitro systems presently employed to study carcinogenesis use mesenchymal or fibroblast cells and transformation is defined by the production of sarcomas. This investigator has been attempting over the past two years to develop a model system in tissue culture for studying epithelial cell transformation. It seems reasonable that epithelial cells will better reflect the malignant changes occurring in vivo, resulting in the production of carcinomas. Moreover, once an epithelial line is established, various contaminants in the atmosphere can be checked for their carcinogenic potential.

In this laboratory, attempts are being made to obtain a line of mouse epithelial cells which is susceptible to transformation by viruses and chemicals. Studies will be made of the steps involved in epithelial cell transformation and the combined effects of chemicals and viruses on this transformation.

The experience and knowledge gained in the cultivation and study of mouse epithelial cells will be used for research on human epithelial cell transformation.

Activation Date: January 1 - March 31, 1975

Current Grant Level: $10,000.

Henry T. Lynch, M.D.
Professor and Chairman
Department of Preventive Medicine
and Public Health
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Creighton University
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Part I : Smoking History in Families with Low and High Cancer Incidences.
Part II: Aryl Hydrocarbon Hydroxylase (AHH): Cancer Genetics.

The aggregation of lung cancer, either site-specific, or in association with cancers of other anatomic sites in families, will be utilized for elucidation of genetic and/or familial factors producing high and low cancer risks. Multiple etiologic factors including cigarette smoking will be studied in context with family history. In addition, aryl hydrocarbon hydroxylase (AHH) will be evaluated in selected high and low cancer risk patients from these kindreds.

Intensive tumor and genealogic documentation will permit critical appraisal of the significance of AHH findings. Inducibility of AHH will be measured in lymphoblasts from patients from low and high risk cancer-prone families in order to determine familial patterns of AHH induction susceptibilities (low, medium and high). Possible association between cancer risk and the inducibility of AHH will be correlated with specific histologic varieties of cancer, their genetic modes of transmission and interaction with cigarette smoking.

The investigators intend to increase their population pool of lung cancer-prone families through contact with cancer institutes and local physicians, and through use of letters indicating their interest in these families to be published in State Medical Journals from seven neighboring Midwestern states. This will provide them with a sizable clinical pool so that specific cancer-prone genotypes might be more readily identified and subjected to critical evaluation by their standard medical genetic protocol, as well as through specialized laboratory studies including AHH.

Activation Date: May 1, 1974

Current Grant Level: Part I : $30,360.
Part II: $29,267.

Hans Meier, D.V.M.
Senior Staff Scientist
The Jackson Laboratory
Bar Harbor, Maine 04609

Oncogenesis in the Rabbit: Genetic Susceptibility, Vertical Transmission of Virus and Environmental Influences.

The investigators believe that three conditions in two related strains of rabbits, hereditary lymphosarcoma and hemolytic anemia which is occasionally associated with thymomagenesis, are caused by a single autosomal recessive gene conferring susceptibility and a vertically transmitted C RNA tumor virus.

To induce, activate and isolate the putative rabbit type C RNA tumor virus five approaches are being used: (1) graft-versus-host reaction in vivo; (2) mixed lymphocyte reaction in vitro; (3) a combined in vitro carcinogen-halogenated nucleotide treatment; (4) cocultivation of rabbit cells with heterologous cells; and (5) tumor induction in vivo with intradermal injections of bovine papilloma virus.

Thus far, they have made three major findings: (1) the likely identity of the genes conferring susceptibility to both lymphosarcoma and immune hemolytic anemia; (2) the probable presence in rabbits of an oncogenic RNA tumor virus; and (3) the occurrence of Coombs' autoantibodies. A putative viral isolate is now being characterized.

Activation Date: July 1, 1974

Current Grant Level: $24,401.

Hans Meier, D.V.M.
Senior Staff Scientist
The Jackson Laboratory
Bar Harbor, Maine 04609

Transplacental Effects of Nitrosocompounds in Inbred Strains of Mice and Rabbits.

The objectives of this investigation are to analyze the transplacental embryotoxic, teratogenic, and carcinogenic effects of two classes of nitrosocompounds, nitrosoureas and nitrosamines, in inbred strains of mice and rabbits. Obviously, these studies are complex, and some narrowing of aims is necessary. Thus, they propose to evaluate the action of the compounds with respect to: (1) their retention, activation (conversion), and degradation; (2) the role of fetal age or gestational days; and (3) the mode of inheritance and number of genetic loci involved in species and strain differences. They feel that detailed definition of these goals is pertinent to the situation in man because (1) malformations, congenital tumors and childhood cancers may have been transplacentally induced; and (2) at least certain types of tumors in adults may be due in part to delayed consequences of carcinogen-exposure during intrauterine life.

Strains of mice chosen for study are: AKR/J, DBA/2J, SWR/J, C57BL/6J, and C57L/J; they differ from one another by genetic origin (except for the last two), type C RNA tumor virus expression, inducibility of aryl hydrocarbon hydroxylase (AHH), and sensitivity to 1-ethyl-1-nitrosourea (ENU)-induced teratogenicity and carcinogenicity among other traits. For studies with rabbits they are primarily focusing on strains III and WH because of their differences in genetic origin and AHH inducibility.

Transplacental exposure is an extremely sensitive method for study of the biological effects of potentially harmful pollutants. Their effects depend upon hereditarily determined differences in susceptibility or resistance of pregnant mothers and embryos, and the stage of fetal (organ) development.

Activation Date: July 1, 1974

Current Grant Level: $25,461.

Microbiological Associates Inc.
4733 Bethesda Avenue
Bethesda, Maryland 20014

Smoke Inhalation Carcinogenesis Study in Mice.

This contract, first of all, is to provide facilities, by renovation, new construction and provision of new equipment, for an extensive study of tobacco smoke inhalation by contrasting strains of mice, under carefully controlled and reproducible conditions, over prolonged periods.

It also provides for completing preliminary studies in preparation for the initiation of long-term experiments.

Such preliminary studies include the following:

CTR 1.1. To determine the amounts of benzo[a]pyrene (B-a-P) which, introduced directly into the lungs of the most susceptible mouse strain (principally the C3H) in various conditions and in various media (such as adsorption on iron oxide) and in several dosage sequences, may induce squamous cell carcinoma of the lung. About 23 experimental groups are contemplated and the observations are to continue as long as any mice survive, to determine the possible appearance of lung cancers or other pathological changes.

CTR 1.2. To collaborate with and assist personnel of the Process and Instruments Corp. in the development of animal holders to be used in such smoke inhalation experiments. These must not only hold the mice in such a way as to facilitate smoke inhalation, but must produce minimal stress, permit rapid and easy placement of mice and allow rapid and efficient cleaning and sterlization.

CTR 1.3. Collaborative studies will be undertaken with the Oak Ridge National Laboratory for determining actual smoke dosage and retention following exposures in the Walton and Process and Instruments Corp. smoke inhalation devices. Exposures of mice of several strains will be carried out at Microbiological Associates and the tissues sent to Oak Ridge for analysis.

CTR 1.4. Collaborative studies will be carried out with Dr. Walter B. Essman, Professor of Psychology and Biochemistry, Queens College, Flushing, N.Y. to measure, pharmacologically, the susceptibilities of various mouse strains to stress, their ability to adapt and the relative stress effects of different types of holding devices. Such determinations are essential since animals, unlike man, inhale smoke under stressful conditions so that the effects of stress may contribute significantly to the overall effects of smoke inhalation experiments.

CTR 1.5. A variety of exposure times and smoke concentrations will be used to determine acute and chronic responses to smokes from reference cigarettes of several types. The effects upon induction of aryl hydrocarbon hydroxylase will be measured.

CTR 1.6. Effects of intratracheal "priming" doses of iron oxide, benzo[a]pyrene and methylcholanthrene administered before or during smoke inhalation will be studied to determine whether inhaled smoke has a "promoter" effect. As many as six groups of experiments, with controls, will be undertaken.

The studies listed are planned for the first part of the contract year while the physical facilities for the more extensive smoke exposure experiments are being completed. At some time during the latter part of the year, extensive experiments on cigarette smoke exposure of mice with contrasting susceptibilities to known carcinogens will be begun.

Activation Date: July 1, 1974

Current Contract Level: $836,143.

Microbiological Associates Inc.
4733 Bethesda Avenue
Bethesda, Maryland 20014

In Vivo Chemical Carcinogenesis.

The objective of this contract is to study the conditions under which lung cancers can be induced and the conditions that may predispose to or reduce the potentiality for the appearance of cancer.

This is a group of several separate studies, some with several parts that have been undertaken to attain the above objective. Some have been completed at the time of writing and all will be completed within the next 6 or 8 months.

CTR 1. Twelve fractions of 1R1 cigarettes, the whole smoke concentrate (WSC) or the reconstituted concentrate (RSC) were injected subcutaneously into mice of the C3H/Mai strain. Fractions and the concentrates were dissolved in either trioctanoin (TOC) or trioctanoin and bees-wax (TOC/B). An equal number of mice were given a sub-effective dose of a cancer-inducing chemical, 3-methyl-cholanthrene (3MC) prior to receiving the fractions to check for promoter effects.

CTR 1A: The same as CTR 1 above except that the fractions of the 1A1 cigarettes were used with the addition of groups of mice receiving larger doses of 3MC.

CTR 1B: The WSC from unknown but different types of cigarettes (coded) were used in the same manner as in CTR 1 above.

CTR 2: To determine the toxicity and the carcinogenicity of several nitrosamines in C57 mice, DMN - dimethylnitrosamine; DEM - diethylnitrosamine; DBM - dibutylnitrosamine; PIP - nitrosopiperidine; PYR - nitrosopyrrolidine. After determining toxicity, smaller doses were given to determine toxicity.

CTR 2A: A repeat of the CTR 2 experiment with the intraperitoneal injection into mice of the C57 strain when 4 to 5 days old to determine the carcinogenic effects. Large groups were used with suitable controls.

CTR 3: The induction of squamous cell carcinoma in the respiratory tract of mice by the intratracheal instillation of 3MC. Intratracheal instillations of 3MC were given groups of mice of 3 inbred strains and one hybrid group. Large doses were given in gelatin in 1, 3 or 6 doses.

CTR 3B: This is a repeat of CTR 3 using mice of the C3H strain and staged doses of 3MC. This experiment should be completed by the end of the current year.

CTR 4: Mice of 2 inbred strains and hybrid groups were given different doses of 3MC subcutaneously to determine the effect of dose on the incidence of cancers of the connective tissues. This experiment was designed to determine the effects of AHH and of the gs antigen on the appearance of tumors.

CTR 5: Relationship between sensitivity to intratracheally instilled 3MC-induced squamous cell carcinomas of the lung and inducibility of AHH activity and the gs antigen expression. Mice of 2 inbred strains, hybrids and back-cross mice were used.

CTR 6: To study the effect of the intratracheal instillation of DEN on the white blood cell response and on the immunocompetence of the treated mice as determined by the acceptance of transplanted tumors.

CTR 7A: The comparison of the pulmonary and the hepatic AHH activity after intratracheal instillation of 3MC. Graded doses of 3MC were given suspended in gelatin.

CTR 9: This experiment was done to compare the 15 WSC used in CTR 1B when they were applied intratracheally rather than subcutaneously. They were given 3 times at 7- to 12-day intervals.

CTR 9A: This is a duplication of the intratracheal application of the 1A1 fractions (see CTR 1A), WCS and RCS according to the schedule of CTR 9 to determine their possible carcinogenicity when applied to the trachea.

CTR 9D: A repeat of 9A above with smaller doses of the fractions.

CTR 9B: This is a duplication by the intrapulmonary application instead of the subcutaneous injection of the WSC of the 15 different cigarettes used in CTR 1B. The WSC were injected into the lung through the intercostal spaces. Pellets of 3MC, B-a-P and DMBA were also given in graded doses to get data on the application of known carcinogens when so applied.

CTR 10: To determine the effect of 1A1 concentrates and fractions on AHH activity. Three fractions seemed to inhibit AHH induction by B-a-P. The fractions were compared to a known AHH inhibitor, 7-8-benzoflavone.

CTR 11: This experiment is designed to determine the induction of pulmonary AHH activity after mice have been exposed to fresh smoke from the 1A1 cigarette. Several strains of mice will be used.

CTR 15, 16, 17: To study the effects of the chlorinated hydrocarbons (TCDD) on AHH induction of mice of different inbred strains. These compounds have been used as herbicides and have extensive contact with man under some conditions. They were widely used in Viet Nam.

CTR 18: To study the toxicity of nitrosamines when instilled intratracheally at 1 or 2-week intervals and to evaluate the carcinogenicity of these substances in mice of an AHH-inducible and a non-inducible strain.

CTR 19: To determine the effect of carrier on the response to known carcinogens. When 3MC was given in TOC:B fewer tumors appeared than when it was given in TOC and the tumors appeared later. Mice of an inducible and a non-inducible strain have been given 3 different cancer-inducing chemicals in graded doses and in the different carriers to determine more about the significance of the carrier.

Activation Date: February 1, 1974

Current Contract Level: $150,000.

Microbiological Associates Inc.
4733 Bethesda Avenue
Bethesda, Maryland 20014

In Vivo and In Vitro Viral-Chemical Carcinogenesis.

This contract is for the support of 18 somewhat separate projects that are designated sequentially from CTR 20 to 37 inclusive. These are as follows:

CTR 20: Effect of diethylnitrosamine (DEN) on pulmonary AHH levels. DMN is one of the substances found in small amounts in cigarette smoke. It is formed when nitrites act on amines, in smoked meats and fish and is abetted by the action of salivary enzymes. It is demethylated by enzymes, demethylases, that may antagonize the hydroxylase enzyme, AHH.

CTR 21: Effects of promoters (substances that will not cause cancer themselves but will make cancers appear when subliminal amounts of a cancer-causing chemical is given) on AHH activity. A substance called a phorbol ester (PE) will be given with very small amounts of a cancer-inducing chemical to determine whether it will increase the incidence of cancer in mice that are susceptible to AHH induction and that are not susceptible.

CTR 22: The effect of Sendai virus infections on pulmonary AHH activity. Sendai virus induces lesions in the lungs of mice that have been mistaken for cancer. These lesions regress however and do not progress. Mice of strains highly susceptible to AHH induction and not susceptible will be compared with respect to response to Sendai and to Sendai virus plus a chemical carcinogen, 3-methylcholanthrene (3MC). Sendai infections have complicated some smoke inhalation experiments.

CTR 23: Further studies on the genetics of AHH induction in mice. Completed experiments have shown that in mice the AHH inducibility is controlled by one gene. The same seems to be true in man. The determinations have been done by studies of increased AHH in preparations made from liver or from white blood cells respectively. This experiment is designed to determine if the skin or lung tissues will also show increased AHH inducibility when properly stimulated by the subcutaneous injection of inducer.

CTR 24: This experiment will again be a long-term experiment much like CTR 23 except that the carcinogen (3MC) will be given through the wind-pipe (trachea). 3MC is not found in tobacco but it will induce lung cancer. This experiment may also be repeated using those substances found in cigarette smoke although in very small amounts.

CTR 25: This is a follow-up on CTR 21 above. The phorbol ester (PE) will be given alone, with 3MC, and 3MC will be given alone, all in the trachea and the effects on lung cancer incidence will be determined. PE is a promoter. This experiment will follow CTR 21.

CTR 26: Viral-chemical carcinogenesis. Sendai virus + 3MC or DEN (diethylnitrosamine, also found in small amounts in cigarette smoke and a powerful cancer-inducing substance under some conditions). The cancer-inducing chemicals will be given and followed at scheduled times by exposure to virus. This will be a rather large experiment and will require careful isolation of animals.

CTR 27: This will be a repeat of CTR 26 above but using PVM (pneumonia virus of mice) instead of Sendai virus. A long-term experiment is planned.

CTR 28: Immunological implications of chemical-virus carcinogens. Age changes occur in the capacity of the body to resist infections and succumb to them. With age some tumor-causing viruses in mice cause expression of tumors. The age changes in the body's defense mechanism will be studied by several methods.

CTR 29: Intratracheal instillation of radioactive labeled cancer cells. Some of these will be tumors that contain and are caused by viruses and others that are presumably not so caused. Attempts will be made to determine how the defense mechanisms of the lungs will be manifested. This is a quite complicated study.

CTR 30: Immune responses of alveolar lymphocytes and macrophages in mice exposed to cigarette smoke and chemical carcinogens. Mice of 3 different strains with and without exposure to cigarette smoke will have the white blood cells washed from their lungs and these cells will be studied with respect to their response to specific stimulations (migration inhibitory factor).

CTR 31: Co-treatment with DEN (see CTR 26) and 3MC (see CTR 24). There may be some antagonism in the body response to these two potent cancer-causing chemicals. The substanceswill be given alone or together by instillation into the trachea and at different instillation schedules. This is another large and long-term experiment.

CTR 32: Effect of vitamin A on AHH pulmonary activity. Large doses of vitamin A seem to prevent cancers that might otherwise be induced by some carcinogenic chemicals. This possibility will be studied using mice of 2 strains and at different levels of vitamin A intake.

CTR 33: Vitamin A in the diet and response to carcinogenic chemicals. Mice will be put on A-deficient diets and adequate diets. Mice of 2 strains will be placed on diets differing in vitamin A content and exposed to at least 2 different cancer-causing chemicals to determine possible differences in response.

CTR 34: The effect of asbestos on pulmonary AHH levels. This will be an attempt to determine whether asbestos will alter AHH activity and the effects of cancer-causing chemicals. The presence of minerals in asbestos may change the activities of some enzymes. A short-term experiment.

CTR 35: Use of tissue culture as an adjunct to in vivo studies. Several methods are used to determine whether cells are cancerous: (1) they may be transplanted to suitable hosts and grow progressively; (2) they may grow continuously in tissue culture in characteristic patterns;or (3) they may show changes in their chromosomes. These studies are designed to establish suitable criteria of cancer.

CTR 36: Use of Balb/c 3T3 mouse cells and chemical carcinogens. This is a lower priority experiment. A well-known cell line (3T3) will be used to try to get cancer in cultured cells.

CTR 37: Use of other cell lines to monitor chemical carcinogenesis. Most cells maintained in culture lose their capacity to induce AHH and if they contain viruses may spontaneously transform to cancer cells. Different cell lines will be studied to determine AHH inducibility and tendency to transform to cancer cells.

Activation Date: July 1, 1974

Current Contract Level: $350,000.

1005075431

Microbiological Associates Inc.
4733 Bethesda Avenue
Bethesda, Maryland 20014

Development of Rapid and Precise Assay Procedures for the Determination of Constitutive and Inducible Levels of Aryl Hydrocarbon Hydroxylase (AHH) in Human Tissues (Especially Blood Lymphocytes) for Application to Population Studies.

The major assays used to detect AHH activity both in vitro and in vivo have been modifications of the original one described by Nebert and Gelboin. The assay is based on the ability to spectrophotofluorometrically detect one of the phenolic metabolites of benzo(a)pyrene (BP), 3-hydroxybenzo(a)pyrene. Advantages of this assay are that it is capable of monitoring both constitutive and induced levels of AHH, is highly sensitive and is relatively inexpensive. Disadvantages are that the low levels of AHH which need to be determined in human studies are close to the sensitivity limits of this assay and extreme care must be taken to avoid day-to-day fluctuations. Another disadvantage is that the assay now takes five days to complete. This definitely limits the number of assays that can be performed.

Three main problems must be worked out before this assay can be used in a large-scale screening study of human population. First, a reproducible source of cells must be made available. Second, procedural modifications must be done so as to lower the rather high non-specific fluorescence observed in the zero-time control cultures. Third, the number of tests capable of being performed must be greatly increased. Since previous studies have shown that the small lymphocyte is capable of giving reproducible AHH inducibility results, this cell is probably the one of choice.

When population tests are underway, the initial study will be concentrated on a lung cancer population. 50-100 lung cancer patients, 50-100 hospital controls and 50-100 non-hospitalized controls will be assayed. Assays will be done at MA at a rate of 20-50 per week. A confirmatory assay* will also be done, but only at a rather low level (about 4-10 per week). This study will also entail a limited questionnaire involvement; including history of cigarette smoking, drug exposure, occupation, etc. Medical verification of the pathological lesion will also be done.

Activation Date: November 1, 1974 to December 31, 1975

Current Contract Level: $108,406.

*-See A-28, Grant 1013, Malcolm C. Pike, M.D.

Microbiological Associates, Inc.
4733 Bethesda Avenue
Bethesda, Maryland 20014

Influence of Exogenous Materials on the Activation of Latent C-Type RNA Viruses.
Contract #2

This program is designed to study the ability of chemicals and fractions of cigarette smoke to activate latent C-Type RNA viruses present in all vertebrate embryonic cells studied, and associated with or present during, the carcinogenic process in chickens and a variety of mammals. Similar C-Type RNA virus particles have recently been identified in a human sarcoma tumor and in a human breast carcinoma.

After a well-evaluated and standardized in vitro assay system for the quantitative measurement of relative carcinogenicity has been developed, that system will be applied to determine the in vitro toxic dose of known chemical carcinogens, tobacco smoke, and selected smoke components for defined tissue-cultured cell lines. Later, the in vitro transformation and viral expression consequent to cell exposures to known chemical carcinogens and to smoke or smoke fractions will be determined. Cells exposed to smoke or smoke extracts for varying periods with and without extraneously added "helper" viruses will be closely watched for cytopathic effect, transformation, viral antigen and, where possible, viral titer by the complement fixation (COMuL) test. To test for transplantability or tumorigenesis, the treated cultured cells will be injected into newborn animals.

Current Contract Level: $250,000.

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Study of Relationship between Susceptibility to Certain Cancers and Aryl Hydrocarbon Hydroxylase (AHH) Activity.

The aims of this project are to establish a repeatable test of AHH inducibility in humans, and to correlate AHH inducibility with susceptibility to cancers of lung and other "chemically induced" human tumors.

The investigators' initial approach is to try to establish a repeatable test of AHH inducibility in human peripheral blood lymphocytes. Failing this, other tissues such as skin fibroblast culture lines will be tested.

At present, the investigators cannot get repeatable results with peripheral blood lymphocytes using 3-methylcholanthrene (3MC) in acetone as the inducer. Different methods of delivering the 3MC to the cells are being tried as well as other inducers.

Activation Date: November 1, 1974

Current Grant Level: $68,581.

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Effect of Cocarcinogens and Tumor Promoters on DNA Repair in Mammalian Cells Susceptible to Chemical Transformation.

The influence of various cocarcinogens and tumor promoters on the repair of ultraviolet light or chemically-induced damage to the DNA of mouse embryo cells in culture will be studied.

Primary and secondary cultures of mouse embryo cells will be exposed to agents known to damage DNA, and the incorporation of radioactive DNA precursors into macromolecular DNA in the presence of the test compounds will be measured under conditions where normal, replicative DNA synthesis is suppressed. Materials to be tested for their effects on DNA repair will include tobacco smoke fractions and pure chemicals known to be present in tobacco smoke.

Activation Date: June 1, 1974

Current Grant Level: $34,645.

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Attempts to Identify the Viral Agent(s) Responsible for Sheep-Lung Adenomatosis and to Transfer this Neoplastic Disease to Rodents.

In recent years it has been shown that spontaneous and induced tumors in rodents contain partially or fully expressed RNA tumor viruses. The reason for this peculiar phenomenon is that the RNA oncogenic virus genome is genetically transmitted to somatic cells in which it remains in a repressed form until environmental factors or oncogenic agents remove the specific inhibitor(s).

Previous work in this laboratory has demonstrated the activation of type-C particles by chemical carcinogens in the lymphoreticular tissues of a low tumor mouse strain. Recently, it has also been observed that hydrazine sulphate and urethane-induced lung tumors in BALB/c mice contain type-C particles and their group-specific antigens; but the particles have not been detected in normal lung tissue of the same mice or in normal lung tissue of untreated mice of the same age. Type-C particles have also been isolated from three tissue culture lines of urethane-induced lung tumors of BALB/c mice.

It may be possible to transfer all the information acquired in the mouse lung tumor system study to the sheep-lung adenomatosis problems. This disease, in fact, shows certain analogies to mouse-lung tumors. Both tumors are derived from type B alveolar cells, both are of low malignancy, as is demonstrated by the slow growth and rare metastatization, and both contain type-C particles. Furthermore, sheep-lung adenomatosis is transmissible by cell-free filtrate of neoplastic lung tissue not only to lambs but also to hoggets and ewes, and a herpes-like virus has been isolated from the adenomatosis affected lungs.

The specific aims of this research project are: (1) to isolate the type-C particles from sheep-lung adenomatosis and to characterize these particles, their group-specific antigens and their biological activity; (2) to isolate the herpes-virus-like particles and their biological properties and, possibly, (3) to transfer the disease to rodents (mice, rats and hamsters).

As about 10% of all human lung tumors are morphologically and biologically similar to those of mice and sheep, this experimental model could have application to humans.

Activation Date: September 1, 1972

Current Grant Level: $15,000.

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Hydrocarbon Metabolizing Enzymes and Lung Cancer.

The carcinogen metabolizing enzyme, aryl hydrocarbon hydroxylase (AHH), can be measure in human subjects using short-term lymphocyte culture. AHH shows genetic variation in a normal human population. Most lung cancer patients have relatively high activities. The objectives of this study are two:

1. To evaluate carefully and seek to improve the assay method;

2. To plan a prospective study of a normal human population in order to evaluate cause/effect relationships between elevated AHH and lung cancer.

To improve the assay method, new and modified techniques of culturing lymphocytes and other blood cells are planned, also improvement in the sensitivity of the enzyme assay. To evaluate the method, double-blind studies are in progress.

The prospective study is in the planning stages, in collaboration with cancer epidemiologists, to determine the optimum population for screening and follow-up.

The double-blind studies are well underway and are showing fairly good reproducibility of the method in most subjects. The assay has been improved in several respects, and is in process of being standardized by research laboratories throughout the country.

The plans for a prospective study are close to being finalized.

Activation Date: January 1 - March 31, 1975

Current Grant Level: $8,750.

Irene Y. Wang, Ph.D.
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Genetic Differences in the In Vitro Metabolism of Chemical Carcinogens by Human and Mouse Tissues.

Several polycyclic aromatic hydrocarbons (PAH) are potent chemical carcinogens on animals tested, and some of the PAH are distributed widely in our environment. PAH need metabolic activation to bind to critical cellular components in tissues and cause cancer. Comparison of genetic differences in the capability of tissues to convert PAH to various derivatives may establish correlations between the metabolite patterns of PAH produced by various tissues and the susceptibility of the species or strains of animals to the carcinogenic action of PAH.

Microsomes from tissues of different strains of mice will be used for studying metabolite patterns of PAH. Metabolites of PAH will be separated by thin layer chromatography, and identified by comparison with authentic compounds, radioscanning, and UV spectroscopy. Metabolites of PAH produced by cultured human lymphocytes will be similarly analyzed.

Strains of mice with high or low inducibility in aromatic hydrocarbon hydroxylase (AHH) activity are being used at the beginning stage of the study. Among the strains of mice studied, either in control or in pretreated mice, there are quantitative differences in the yield of various metabolites of benzo(a)pyrene, which is one of the potent carcinogenic PAH.

Activation Date: January 1, 1975

Current Grant Level: $65,926.

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Histochemistry of Epithelial Mucins in Carcinoma of the Lung.

Grant #639R1

The proposed study will apply a series of modern histochemical techniques for the demonstration of mucins and mucopolysaccharides to a series of cases of adenocarcinoma, and the subgroup bronchiolo-alveolar carcinoma, of the lung. The same techniques will be applied to a series of normal lungs for suitable control material. This information will then be correlated with follow-up data on the patients which is being obtained from the tumor registry of this institution.

An attempt will be made to answer the following questions:

1. What proportion of the cases of this type of pulmonary neoplasm produce mucins or mucopolysaccharides?

2. What variety of mucopolysaccharides is produced?

3. Is the type of mucin or mucopolysaccharides produced homogeneous in a given case and is it the same for tumors of the same histologic type?

4. Do carcinomas produce a mucin qualitatively different from the parent tissue?

5. Is there a correlation between mucin production and clinical course?

Current Grant Level: $14,885.

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Avian Tumor Viruses in Mammalian Hosts.

1. Avian RNA tumor viruses transcribe all or part of their genome into DNA. The mechanism and extent of this transcription will be studied. Experiments will also be carried out to determine the amount of viral RNA or DNA present in the cell and to define genetic relatedness of various avian RNA tumor viruses by nucleic acid hybridization.

2. Avian tumor viruses contain sufficient genetic information to code for 30 to 40 proteins. Only seven proteins occur in the virus particle itself, the remainder must be nonstructural viral proteins synthesized in the infected cell. The search for such nonvirion proteins will be conducted after the development of suitable in vitro tests. Studies on the function of these nonstructural proteins in virus induced transformation and virus replication will be carried out.

Activation Date: January 1, 1971

Current Grant Level: $27,071.

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Cigarette Smoke and Polycyclic Hydrocarbon Metabolism in Rat and Mouse Lung and Kidney.

Potent inducers of aryl hydrocarbon hydroxylase are present in cigarette smoke. In rats and mice, they specifically increase the lung and kidney enzymatic activity. Certain biochemical characteristics of this phenomenon make the investigators believe that the responsible inducing substances in the cigarette smoke might be different from the polycyclic hydrocarbons.

The researchers' immediate goals will be (1) to identify those inducers and (2) to further characterize the biochemical mechanism of their action compared to other well-known inducers. Their longer range program includes a detailed biochemical study of the various enzymes (aryl hydrocarbon hydroxylase, epoxyde hydratase, glutathione transferase) involved in the polycyclic hydrocarbon metabolism, as well as a molecular approach of the early biochemical modifications produced in the target tissues (lung and kidney) by the polycyclic hydrocarbons.

Presently, the investigators are studying in more detail the mechanisms of AHH induction by cigarette smoke condensate fractions mainly using different cell lines in culture as models. They are also analyzing the action of cigarette smoke on the activity of other enzymes of polycyclic hydrocarbon metabolism, (epoxyde hydratase and glutathione transferase), as well as on the binding of polycyclic hydrocarbon metabolites to the macromolecules of rat and mice (inducible and non-inducible) lung and kidney.

In the very near future, they will also further analyze the chemical composition of the smoke condensate fractions in relation to their effect on the drug metabolizing enzymes. High pressure liquid chromatography will be used to further separate the components of those fractions. Gas liquid chromatography coupled to the mass spectrometry will be utilized for precise analytical work and identification of the main constituents.

Activation Date: June 1, 1976

Current Grant Level: $47,000.

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Oncornaviral Gene Expression in Normal and Malignant Tissues.

Study of the major oncornavirus glycoprotein has continued. The object is to determine the degree of polymorphism which accompanies endogenous expression of the provirus. Tryptic peptides have been analyzed for four different ecotropic and four different Xenotropic viruses. The pattern is different in each case. The gp70 from the serum of high expression mice was isolated and its tryptic peptides studied. This protein seems to be coded for by the Xenotropic genome.

Activation Date: July 1, 1976

Current Grant Level: $65,000.

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Possible Genetic Determinants of Chemical Carcinogenesis.

The objective of this project is to develop a model system in vitro for studying carcinomas. Possible interplay of endogenous viruses and chemicals in cocarcinogenesis will be investigated here.

An epithelial cell line has been derived from the liver of NZB mice. The characteristics of this line and its sensitivity to chemical transformation will be studied. The genetic basis for viral and chemical carcinogenesis will be studied by breeding experiments using NZB, 129/J, and SWR mice. NZB mice spontaneously produce significant titers of xenotropic virus. 129 and SWR produce very little. The possible interplay of X-tropic virus production and chemical induction of transformation will be examined by using F1, F2 and backcross generations.

(NZBx129)F_1 produce significant titers of infectious xenotropic virus whereas 129 mice alone do not. Testing of the NZBxSWR and the backcross generations for virus production is in progress. Concomitantly, these mice will be tested for susceptibility to tumor induction by 3-mercaptoethanol.

Activation Date: April 1, 1976

Current Grant Level: $71,900.

Henry T. Lynch, M.D.
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Part I: Smoking History in Families with Low and High Cancer Incidence.
Part II: Aryl Hydrocarbon Hydroxylase (AHH): Cancer Genetics.

Lung cancer, either site-specific or in association with cancers of other anatomic sites in family pedigrees, will be used for evaluation of genetic and/or familial factors which are thought to produce high and low cancer risks. Various etiological factors, such as cigarette smoking, will be analyzed in context with family history. Furthermore, aryl hydrocarbon hydroxylase (AHH) will be examined in selected high and low cancer risk patients from these kindreds.

Extensive tumor and genealogic documentation will allow critical appraisal of the significance of AHH findings. AHH inducibility will be measured in lymphoblasts from patients with low and high risk cancer prone kindreds for the purpose of determining familial patterns of AHH induction susceptibilities (low, medium and high). Possible associations between cancer risk and the inducibility of AHH will be correlated with various histologic varieties of cancer, their genetic modes of transmission, and interaction with cigarette smoking.

The investigators plan to expand their population pool of lung cancer prone families through association with cancer institutes, community physicians, and through utilization of letters indicating their interest in these kindreds to be published in State Medical Journals from seven neighboring midwestern states. This will grant a sizable clinical pool so that specific cancer prone genotypes might be more readily identified and subjected to critical evaluation by the standard medical genetic protocol, as well as through specialized laboratory studies including AHH.

Activation Date: May 1, 1976

Current Grant Level: $75,000.

Hans Meier, D.V.M.
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The Jackson Laboratory
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Oncogenesis in the Rabbit.

The investigators aim to show that lymphosarcoma development in strain WH rabbits and immune hemolytic anemia associated with thymomagenesis in strain X (which is genetically related to strain WH) are due to a single gene which confers susceptibility to both conditions, and to an hereditary type C RNA tumor virus genome. There is evidence for both a high molecular weight (70S) RNA and an RNA-directed DNA polymerase (RDDP) associated with a particular banding in the density region of a type C RNA virus. The RDDP prefers poly (rA) · $(dT)_{12-18}$ and poly (rC) · $(dG)_{12-18}$ and other synthetic templates and also utilizes viral 70S RNA.

Activation Date: July 1, 1976

Current Grant Level: $27,333.

Hans Meier, D.V.M.
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The Jackson Laboratory
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Transplacental Effects of Nitrosocompounds.

The investigators are analyzing the transplacental carcinogenic effects of nitrosocompounds in inbred strains of mice and rabbits. These effects depend greatly upon the hereditary differences in susceptibility or resistance of pregnant mothers and embryos, and the stage of fetal (organ) development. Examples are the transplacental induction of primary renal tumors in rabbits, the transplacental effects of diethylnitrosamine in mice as related to strain of mice (genotype) and day(s) of administration during pregnancy, etc.

Activation Date: July 1, 1976

Current Grant Level: $28,584.

Microbiological Associates
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Development of In Vitro and In Vivo Model Systems for the Study of Lung Chemical Susceptibility and Carcinogenesis.

Section A: "In Vivo Model Systems Studies"

At the beginning of the new contract year on July 1, 1976 the following studies will be in progress and be carried over for completion:

CTR 47: Subcutaneous Carcinogenic Bioassay of CSCF Material
CTR 49: Intratracheal Carcinogenic Bioassay of CSCF Material
CTR 50: Serial Sacrifice of BaP Intratracheal-Treated Mice for Definition of Histopathological Lesions
CTR 51: Serial Sacrifice of MCA Intratracheal-Treated Mice for Definition of Histopathological Lesions

In an experimental series to be developed in 1976-79, primary emphasis will be to define the role of initiators, promoters and cocarcinogens. Such a model would be developed for the purpose of testing smoke and its constituents for their possible role in the carcinogenic process. Model experiments using known chemicals with known roles either as carcinogens, cocarcinogens, etc. would be performed to define this system; and tobacco smoke constituents would be integrated to test their effects in the systems developed.

Section B: "In Vivo and In Vitro Model Systems Studies"

The contractees propose to use a combination of in vivo - in vitro model systems to answer the following questions: Can tobacco-related products be analyzed for their tumor promoting effects in a cell culture system? Can these products be analyzed for their mutagenic, carcinogenic, or cytotoxic effects in an in vitro model system? Can these culture systems be made more sensitive, more discriminatory, and capable of determining the molecular mechanism for PAH-induced carcinogenesis, mutagenesis, and cytotoxicity?

The combination in vivo - in vitro model systems include two mutagenesis systems (one bacterial and one mammalian), and three cell transformation systems (BALB/3T3, 10 T 1/2 CL 8, and Syrian golden hamster fetal (SHE) cells). The biological effects to be examined, mutagenesis and cell transformation, can be quantitated in these model systems.

Activation Date: July 1, 1976

Current Contract Level: $350,000.

Microbiological Associates
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Smoke Inhalation Carcinogenesis Studies in Mice.

The work on CTR-22 will consist of two primary aspects during the new contract year (July 1976-June 1977): Dosimetry and Inhalation Studies.

The proposed dosimetry studies in collaboration with Oak Ridge National Laboratory include study on the Walton and SEM II as summarized below.

With Walton Smoking Machine

1. Completion of Present Studies
2. Deposition as a Function of Adaptation
   a. Non-Adapted
   b. Two cigarettes
   c. Short-term Adaptation 10 Cigarettes/Day
   d. Maintained 1 Month on 10 Cigarettes/Day

With SEM II

1. Determine Experimental Design for Dosimetry Studies
2. Exposure Time
3. Smoke Concentration
4. Smoke Flow Rate
5. Age of Smoke
6. Relative Humidity
7. Animal Contaminant System
8. Characteristics of Various Cigarette Types

Inhalation studies on both the Walton and SEM II will be conducted during this contract year. The Waltons are being used to carry out a study in which carcinogen loaded cigarettes will be used. The SEM II machines will be used to carry out acute toxicity studies, as well as tolerance/adaptation measurements.

Activation Date: July 1, 1976

Current Contract Level: $900,000.

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5221 River Road
Bethesda, Maryland 20016

Research Services to be Performed in Support of Grant 1011 (Jay A. Levy, M.D.) and Grant 1005 (Irene Y. Wang, Ph.D.).

In support of Dr. Levy's work, Microbiological Associates will:

(1) Provide about 850 animals from crosses involving the 129/J and NZB strains of mice.

(2) Perform partial splenectomy as required for virus isolation.

(3) Take sera at predetermined intervals and test for antibody titers against the xenotropic virus.

(4) Treat animals with 500 µg MCA and palpate for subsequent tumor formation.

(5) Try to determine the role of the xenotropic and ecotropic viruses in MCA-induced tumorigenesis.

(6) Perform AHH assays on a representative number of animals.

In support of Dr. Wang's work, Microbiological Associates will supply frozen specimens of liver and lung of mice of several different strains and hybrids for Dr. Wang to determine and quantitate the metabolic deviates of benzo(a)pyrene in induced and noninduced tissues.

Activation Date: July 1, 1976

Current Contract Level: Levy $34,250.
Wang $ 5,926.

Kenneth Paigen, Ph.D.
Director, Molecular Biology Department
Health Research, Inc.
Roswell Park Division
666 Elm Street
Buffalo, N.Y. 14263

A Genetic Test of Glucoronidase in Bladder Cancer.

Levels of β-glucuronidase, α-galactosidase, β-galactosidase and β-hexosaminidase are being evaluated in the urine of normal individuals, bladder cancer patients, bladder cancer patients in remission and relatives of bladder cancer patients. The object is to determine whether the elevated urinary β-glucuronidase levels characteristic of this disease are a cause or a consequence of the disease. This distinction will be made by a genetic analysis comparing the relatives of bladder cancer patients with their parents and with an appropriate control population.

Activation Date: July 1, 1976

Current Grant Level: $54,112.

Ronald E. Rasmussen, Ph.D.
Associate Research Physiologist
Department of Community and Environmental Medicine
California College of Medicine
Irvine, California 92717

Effect of Cocarcinogens and Tumor Promoters on DNA Repair in Mammalian Cells Susceptible to Chemical Transformation.

The objectives of this project are to determine whether cigarette smoke condensate fractions or other tumor promoters may act through the inhibition of DNA repair, and to determine whether exposure of animals to whole smoke may result in reduced DNA repair capacity.

Cell cultures derived from experimental animals and humans will be exposed to smoke condensate fractions and the effect on DNA repair will be examined. Tissues of exposed animals will be examined for their ability to respond to challenge by agents which are known to damage DNA, including alkylating agents and hydrocarbon carcinogens. Inbred strains of mice will be studied to determine whether genetic differences exist in the ability to carry out DNA repair.

Activation Date: July 1, 1976

Current Grant Level: $45,562.

David W. Talmage, M.D.
Director
Webb-Waring Institute
University of Colorado Medical Center
4200 East Ninth Avenue
Denver, Colorado 80220

The Role of Macrophage Induced Factors in Cancer Immunity.

The aims of this project are:

(1) To purify and identify the factors in supernatants of mixed cultures of macrophages and lymphocytes which suppress the activation and proliferation of thymus derived lymphocytes (T cells).

(2) To determine by which cell(s) the suppressing factors are produced.

(3) To compare these factors with those secreted by several tumor cell lines.

(4) To determine whether the suppressor factors act at a particular point in the cell cycle.

The long-term goal of the project is to understand how the activation and function of cytotoxic T-lymphocytes are controlled. The investigators will begin with an attempt to understand suppression. Then they can determine whether activation is a reversal of suppression and perhaps apply this knowledge to the indication of immunity to cancer cells.

Activation Date: July 1, 1976

Current Grant Level: $25,000.

George Wolf, B. Sc., D.Phil.
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Effect of Carcinogens on Glycoprotein Synthesis.

Having established that the tracheal enzyme from rats which effects transfer of galactose from UDP-galactose to glycoprotein is severely depressed in vitamin A-deficiency, the investigators propose to determine if the same happens to the enzyme in the process of carcinogenesis. Therefore, they will investigate the:

(1) effect of methylcholanthrene instillation (at high dose levels) on galactosyl transferase in vitamin A-deficient and normal rat respiratory tract.

(2) effect of different divalent metals on the activity of tracheal galactosyl transferase, alone and in competition with Mn^{++}, in MC-treated and untreated rats.

The researchers will also continue their current research on the properties of tracheal galactosyl transferase after solubilization and the mechanism of its activation by in vitro addition of vitamin A.

Activation Date: July 1, 1976

Current Grant Level: $24,198.

Tob. Smoke Stays

1005075454

Bio-Research Institute, Incorporated
9 Commercial Avenue
Cambridge, Massachusetts 02141

An Expanded Study of Mouse Skin Exposure to Whole Fresh Smoke Compared to Skin Painting.

Contract #4

This study involves exposure of the skin of 220CAF$_1$ mice to cigarette smoke jets emanating from a smoking machine, and to certain control conditions. Eight groups of mice are to be exposed three times a week to jet smoke alone, or with solvent or promoter chemicals added singly or together. Appropriate controls with the chemicals alone, or with skin carcinogens, are to be included. Completion of this experiment will include histopathological study of all the exposed skin areas. In addition, a pilot exposure experiment will be run with hairless mice. A positive control group will be investigated to determine the susceptibility of hairless mice to skin tumor induction.

Current Contract Level: $60,000.

Bio-Research Consultants, Incorporated
9 Commercial Avenue
Cambridge, Massachusetts 02141

The Determination of the Usefulness of the Golden Syrian Hamster as Model Animal for Inhalation Studies. Contract #5

Animals of two inbred strains of Syrian hamster -- (1) "cancer susceptible" and (2) "hardy" or "cancer resistant" -- will be exposed to inhalation of whole smoke and of the gaseous phase of smoke. LD_{50} values will be determined for both strains, using no less than five levels per dose with 24 animals per level. Maximum-tolerated dose will be determined for both strains on a chronic basis, including duration of exposure at various degrees of dilution of smoke with air and study of blood CO levels. After these determinations have been made, long-range lifetime experiments will be initiated. During the first year a special study will be made on hamsters exposed to smoke inhalation. At the conclusion of the study, all animals will be autopsied, all organs will be inspected, and histological sections will be prepared.

A second study will be done in order to ascertain that dose of hydrocarbon which, when intratracheally injected, will not produce tumors but could be used as an initiating dose in experiments testing for any co-carcinogenic effect of intratracheal cigarette smoke condensate and, later on, inhaled smoke. Four groups of 25 BIO 15.16 hamsters will be used.

Current Contract Level: $75,000.

T. Timothy Crocker, M.D.
Department of Medicine
University of California
San Francisco Medical Center
San Francisco, California 94122

Biologic Activity of Tobacco Tar on Respiratory Mucosa of Rodents, Canines and Primates in Organ Culture; A Histologic and Autoradiographic Study.

Grant #572R1

Organ cultures of trachea, bronchi and lungs from unborn or newborn hamsters, dogs and monkeys will be exposed to crude tobacco products or their fractions. Cultures will be fixed at intervals and will be labeled with tritiated thymidine just before fixation. Sections will be examined to learn whether an abnormal appearance of cells or a change in cell growth rates has been produced. The effect of tobacco products, if any, will be compared with effects of known carcinogens or with the abnormal states present in bronchial epithelium of human subjects with lung cancer. In addition, the extent of abnormality will be compared in respiratory epithelia of the three animals under study to learn whether any or all are susceptible to the action of tobacco products. Comparative studies of this type will help to determine whether common laboratory animals (hamster and dog) are useful in predicting the response of the respiratory tract of primates (monkey and man) exposed to such materials.

Current Grant Level: $61,584.

Cecile Leuchtenberger, Ph.D.
Department of Cytochemistry
Swiss Institute for Experimental Cancer Research
Lausanne, Switzerland

Comparative Cytochemical, Cytological and Histological Studies of Early Effects of Cigarette Smoking (Whole, Gas Phase, Constituents) in Mice and in Tissue and Organ Cultures from Mice and Humans. Grant #413-AR1

The main purpose of the proposed research project is an investigation of early interaction between native cigarette smoke or some of its constituents and intracellular metabolism. The plan to examine this relationship simultaneously in the living animal (mouse) and in mouse and human tissue and organ cultures at the cell level itself has two main specific aims:

a) Is the response of cells and tissues to exposure of cigarette smoke the same or different in vivo and in vitro?

b) Are very early alterations in cell metabolism detected and what is their relation to the occurrence of pathological alterations, including tumors, observed after long-term exposure of mice to chronic inhalation of whole cigarette smoke or its gas phase?

Such an experimental approach which places emphasis on assessment of very early sequential events at the cell level itself may help in the elucidation of the complex problem regarding the roles of cigarette smoke and other factors, such as virus infections, for etiology and pathogenesis of malignant tumors.

Current Grant Level: $32,988.

Mason Research Institute
Harvard Street
Worcester, Massachusetts 01608

The Mechanical and Biological Evaluation of Smoking Machines. Contract #1.

The mechanical and electrical features, the puff characteristics, and parameters related to animal exposure will be evaluated for several smoking machines and for both whole smoke and gaseous phase. Also to be obtained will be some analytical data on the gaseous and particulate phases of cigarette smoke generated from each apparatus.

Mice will be exposed to various doses of smoke to establish dose levels for 56 days of continuous treatment. Preliminary biochemical measurements and a minimum of preparative histology and histochemistry will be carried out on these animals.

Current Contrace Level: $119,329.

Mary Stearns Parshley, Ph.D.
Columbia University College of Physicians and Surgeons
630 West 168th Street
New York, New York 10032

Effect of Tobacco Smoke on Normal Mouse Lung Tissue. Grant #630-A

The objective of this study is to evaluate the effect of chronic inhalation of tobacco smoke on the lung tissue of normal mice. The study will be made by tissue culture techniques which provide simple and inexpensive means for the observation of the effects of tobacco smoke on growth rate, metabolism, physiology and cytology, and genetic character of normal lung cells. Studies of the lungs of animals which have inhaled smoke should be of the greatest significance in the detection of differences from the normal lung which may indicate the development of precancerous or cancerous changes in the respiratory epithelium resulting from the smoking of tobacco. By means of tissue culture the response of cells isolated from systemic conditions can be observed directly both in the living state and following histological procedures. Small volumes of pure treated and untreated respiratory epithelial cells can be analyzed and compared by biochemical and histochemical procedures. By using two different approaches, each with different advantages, it should be possible to determine whether chronic inhalation of tobacco smoke results in changes in the lung indicative of the development of malignancy.

Current Grant Level: $34,812.

William A. Carter, M.D.
The Johns Hopkins University School of Medicine
725 North Wolfe Street
Baltimore, Maryland 21205

Oncogeny and the Antiviral Action of Interferon. Grant #694R1

The objectives of these studies are to define unique steps in viral replication in the hope of developing specific antiviral drugs. Current objectives include:

(a) Purification and characterization of the subunit structure of human interferon.

(b) Determining structural requirements of ribopolymers for the induction of human interferon.

(c) Determining molecular loci of action of interferon.

(d) Determining molecular events necessary for transformation of cells by the oncogenic virus SV_{40}.

Current Grant Level: $29,193.

John E. Craighead, M.D.
Department of Pathology
University of Vermont
Burlington, Vermont 05401

Epithelial Cell Transformation and Carcinoma Induction by "C" Type RNA Viruses.
Grant #550-AM

Although "C" type RNA viruses grow in many different types of epithelial cells, their role in the induction of carcinomas in animals is not clearly defined. If "C" type viruses are indeed a major cause of cancer of all types in man then transformation of epithelial cells by these viruses and the establishment of transplantable carcinomas should be possible. I believe insufficient attention has been directed to this question. The studies to be undertaken in this investigation are designed to elucidate the biological factors influencing epithelial cell transformation and the induction of carcinomas by "C" type viruses.

Current Grant Level: $25,439.

T. Timothy Crocker, M.D.
Professor of Medicine
Cancer Research Institute
University of California
San Francisco, California 94122

A Study of the Role of Chemicals and Viruses in Neoplastic Transformation of Mouse and Hamster Respiratory Epithelial Cells. Contract #8

One theory of carcinogenesis suggests that spontaneous leukemias and sarcomas and those induced by irradiation and chemicals result from activation of an indigenous "latent" virus. Observations by other investigators have established the fact that C-type RNA viruses are associated with tumors of mesenchymal cells. In this study, the experimenters propose to extend these observations to mouse and hamster respiratory epithelial cells. To this end, they will examine the possibility that chemicals alone or in concert with C-type RNA viruses transform respiratory epithelial cells to produce, upon subinoculation in animals, tumors of squamous or anaplastic epithelial cells analogous to bronchogenic carcinomas of man. If it appears that chemicals alone transform cells, the possible emergence of indices that an RNA virus was indigenous in the epithelial cells will be studied. Specifically, this viral carcinogenesis study has two aims:

1. To produce neoplastic transformation of mouse respiratory epithelial cells in cell culture.

2. To test neoplastic transformation of hamster respiratory epithelium in organ culture.

Current Contract Level: $53,125.

Hans Meier, D.V.M.
The Jackson Laboratory
Bar Harbor, Maine 04609

Oncogenesis in the Rabbit: Genetic Susceptibility, Vertical Transmission of Virus and Environmental Influences. Grant #758

In all, probably fewer than 30 cases of lymphoreticular tumors of rabbits have been reported since the earliest description of a case of visceral lymphosarcoma in 1914. We have observed over 30 cases of lymphosarcoma in a small breeding colony of Wirehair (WH) rabbits within a few years, and affected animals of both sexes were found in each of several generations. Although they descend from a common male sire, many have additional common ancestors. Because of the unusual case aggregation of lymphosarcoma in WH rabbits, we wish to investigate the host genetic factors conferring susceptibility to lymphosarcomagenesis, the mode of inheritance or transmission, the possibility of a vertically transmitted virus, and the environmental influences which may modify incidence and pathogenesis of lymphosarcoma.

Another strain of rabbits, strain X, which is genetically related to the WH strain, is characterized by a high incidence of immune hemolytic anemia and thymoma. We wish to determine the pathogenic bases of both immune hemolytic anemia and thymoma, and investigate their possible etiological relationship.

We want to find out the mode of inheritance or transmission of immune hemolytic anemia and thymoma in strain X rabbits so that we may evaluate the possibility of a common hereditary basis of all conditions in both strain X and WH, since the two strains are genetically related. It may be that the various clinical or phenotypic expressions derive from differences in the genetic background of the two strains.

Current Grant Level: $23,206.

Richard A. Lerner, M.D.
Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

Studies on Persistent Viral Infection. Grant #766

A continuous line of murine lymphocytes persistently infected with Moloney virus will be under investigation in our laboratory. In agreement with others, we have shown that maximum synthesis of viral antigens occurs during the G_1 phase of the lymphocyte cell cycle. This implies that there is a limited period in the cell cycle during which the viral genome can be expressed, and is an example of host control of virus function. The experiments to be carried out are designed to examine the molecular events involved in this control.

Current Grant Level: $29,345.

John W. Parker, M.D.
University of Southern California School of Medicine
2025 Zonal Avenue
Los Angeles, California 90033

Mechanisms of Suppression of Cellular Immunity by Carcinogenic Hydrocarbons.
Grant #763

A study of the effect of hydrocarbon carcinogens administered *in vivo* and *in vitro* upon the events of *in vitro* cellular immune responses involving macrophages and lymphocytes, as determined by measuring antigen uptake and processing, lymphocyte transformation and proliferation, production of immunologically active substances, and target cell destruction.

Current Grant Level: $20,815.

William Regelson, M.D.
Department of Medicine
Medical College of Virginia
Richmond, Virginia 23219

The Relationship of Phagocytosis to Resistance to Microorganisms and Tumor Induction and Growth. Grant #715R1

Anionic polyelectrolytes suppress the growth of transplanted tumors and increase host resistance to viral-induced neoplasms and protect mice again pneumococcal and cryptococcal infection. This is possibly related to the induction of interferon and enhancement of immunological response and is associated with both a blockade and stimulation of reticuloendothelial function as measured by the phagocytic clearance of colloidal carbon, tagged lipid emulsion and sheep red cells.

We are attempting to see what factors are involved in relation to the enhancement of host defenses against tumor growth and viral, bacterial and fungal infection. The action of polyanions (pyran copolymer) (NSC 46015) and synthetic polynucleotides (Poly:IC) as related to interferon production, phagocytosis and antibody response is under study. We are fortunate in having ^{14}C tagged pyran copolymer and can thus correlate physiologic effects as related to host resistance with distribution and metabolism of this polyanion. In addition, we are studying the effect of other macromolecules including polycations, as well as chronic infection (e.g. BCG), as well as colloidal particulates (lipid emulsion) correlating alterations and susceptibility to tumor growth and infection with phagocytosis, interferon induction and immunologic response.

Knowledge of the functional interrelationships as determined by these assays will give us a better understanding as to what factors might be involved and utilized in conjunction with effective chemotherapy against cancer and/or infection. In this regard we have found that the lethality of cryptococcal infection and pneumococcal infection can be aborted by the synthetic polyanion, pyran copolymer (NSC 46015), which is currently in clinical trial. Preliminary evidence suggests that this compound is excreted and/or metabolized and thus we are also attempting to develop new techniques for its administration including nebulization as a method which might be used effectively to protect animals from infection and tumor growth.

Current Grant Level: $20,030.

Peter K. Vogt, Ph.D.
Department of Microbiology
University of Washington School of Medicine
Seattle, Washington 98105

Avian Tumor Viruses in Mammalian Hosts. Grant #733

Infection of mammalian cells with avian tumor viruses will be used to study host-controlled variation of RNA tumor viruses and virus-controlled, nonstructural antigens in transformed cells.

Current Grant Level: $49,269.

CARDIOVASCULAR

1005075470

Richard J. Bing, M.D.
Director of Cardiology and Intramural Medicine
Huntington Institute for Applied Medical Research
734 Fairmount Avenue
Pasadena, California 91105

Inhibition of Cholesterol Uptake by Arteries In Vitro and In Vivo.

This work is concerned with (1) the mechanism of the inhibitory action of 7-ketocholesterol and (2) its possible inhibitory effect on the arterial uptake of cholesterol in macaca fasicularis, a subhuman primate.

The first portion of the project deals with the effect of lipoprotein on inhibition of cholesterol uptake by the arterial wall as influenced by 7-ketocholesterol on the physical characteristics of plasma lipoproteins. It also is concerned with the question of whether plasma proteins are essential in the inhibitory effect of 7-ketocholesterol. The second part deals with efforts to obtain high blood levels in subhuman primates and the effect of this on inhibition of cholesterol uptake by the arterial wall in these animals. Several possibilities will be examined to raise plasma levels of 7-ketocholesterol.

Activation Date: July 1, 1976

Current Grant Level: $48,764.

H. Fred Downey, Ph.D.
Assistant Professor of Physiology
Director, Cardiopulmonary Research
Cardiopulmonary Institute
University of Texas Health Science
Center at Dallas
5233 Harry Hines Boulevard
Dallas, Texas 75235

Effects of Tobacco Smoke and Nicotine on Coronary Collateral Blood Flow.

The effects of inhaled tobacco smoke on coronary hemodynamics and regional blood flow will be investigated in dogs with acute or chronic coronary artery occlusion.

Exposure to tobacco smoke will be accomplished by placing a lighted cigarette in the air intake line of the respirator. The distribution of blood flow in myocardium and in other tissues will be computed from the uptake of differently labeled radioactive microspheres (7-10 μ) administered during control and experimental conditions. Chronic coronary occlusion will be accomplished by surgically implanting an ameroid constrictor on the anterior descending coronary artery, six to eight weeks before the acute study.

Alpha and beta adrenergic blocking agents will also be administered to determine their effects on the hemodynamic and blood flow response to tobacco smoke.

Activation Date: July 1, 1976

Current Grant Level: $18,222.

Reginald G. Mason, M.D., Ph.D.
Pathologist-in-Chief
Memorial Hospital
Pawtucket, Rhode Island 02860

Effects of Nicotine on Interactions of Platelets and Endothelial Cells.

The overall aim of this research project is to delineate the effects of nicotine on interactions of platelets and endothelial cells. The investigators have shown that nicotine enhances aggregation of platelets induced by adenosine diphosphate. They propose to continue study of the effects of nicotine on the growth of endothelial cells in tissue culture.

In preliminary experiments, it has been found that nicotine inhibits the growth of cultured endothelium. In addition, the researchers propose to look at effects of nicotine on the enhancement of growth of tissue culture endothelial cells produced by addition of blood platelets. The effect of nicotine on the adhesion of platelets and other blood cells to endothelial cells in culture will be studied. Platelets and other blood cells will be applied to monolayers of cultured endothelial cells by controlled centrifugal forces, and then centrifugal force will be used to remove the less firmly adherent cells. Adherence of platelets and other blood cells to endothelial cells will be quantitated by direct microscopic count. Further, the possible effects of nicotine on the contraction of endothelial cells in culture will be studied. Such contraction of endothelial cells is brought about by addition of numerous substances including thrombin.

Finally, the investigators propose to determine the effects of nicotine on the action of an endothelial cell inhibitor of platelet function. This inhibitor has been investigated in the past and has been found to be a small but labile molecule. They are now able to stabilize this activity from endothelial cells and plan to investigate possible effects of nicotine on this inhibitor.

Activation Date: July 1, 1976

Current Grant Level: $29,350.

Louis A. Soloff, M.D.
Division of Cardiology
Temple University Health Sciences Center
3401 North Broad Street
Philadelphia, Pennsylvania 19140

Role of Lecithin: Cholesterol Acyltransferase (LCAT) in Cholesterol Metabolism in Health, Disease and During Smoking.

The investigators have finally been able to produce antibodies to human plasma lecithin cholesterol acyltransferase. Once they are able to establish the range of normal values of LCAT in human plasma or serum, they shall be able to interpret LCAT values in various lipid disorders with particular reference to atherosclerosis. They shall also be able to determine the effects of substrates, activators and inhibitors on the cholesterol esterifying activity of plasma which contains a known quantity of LCAT. Such information should be not only of important diagnostic value and possibly of therapeutic value, but also should increase the researchers' insight into lipid metabolism both in health and in lipid disorders of activity of plasma with particular reference to atherosclerosis.

Activation Date: July 1 - December 31, 1976

Current Grant Level: $34,346.

Stephen F. Vatner, M.D.
Associate Professor of Medicine
Harvard Medical School
Peter Bent Brigham Hospital
721 Huntington Avenue
Boston, Massachusetts 02115

Nicotine Induced Reflex Activation.

The goal of this research program is to delineate the effects of nicotine on regional blood flow distribution and regional resistances in conscious, instrumented dogs. In particular, the effects mediated through nicotine stimulation of peripheral chemoreceptors and subsequent stimulation of thoracic wall stretch receptors will be examined.

At operation using pentobarbital, Na anesthesia, electromagnetic or Doppler flow probes will be placed on the aortic root and left circumflex coronary mesenteric, renal and iliac arteries, and catheters will be implanted in the aorta to measure pressure and in the common carotid artery in order to deliver small quantities of nicotine locally to the carotid chemoreceptors. After control recordings at rest and with the dogs in the basal state, small doses of nicotine (2-40 µg/kg) will be injected intravenously as a bolus. The resultant effects of chemoreflex stimulation and accompanying hyperventilation will be examined on blood flows and resistances in the mesenteric, renal and iliac beds. The efferent mechanisms will be analyzed by repeating this procedure after selective and combined blockades of beta adrenergic receptors with propranolol, 1 mg/kg, alpha adrenergic receptors with phentolamine, 1 mg/kg, and cholinergic blockade with atropine, 0.2 mg/kg. In order to separate systemic effects of nicotine from its actions to stimulate chemoreceptors, nicotine will be injected in a bolus into the implanted carotid arterial catheters in small doses (0.2-0.4 µg/kg). The effects of intracarotid nicotine will be examined on the same parameters as mentioned above, before and after selective and combined autonomic blockades.

Preliminary studies indicate that the reflex vasodilation responses from nicotine stimulation of chemoreceptors and pulmonary inflation reflexes are most prominent in the coronary bed and less evident in the mesenteric, renal and iliac circulations. In fact, when respiration is held constant, striking vasoconstriction is observed in the mesenteric, renal and iliac circulations.

Activation Date: July 1, 1976

Current Grant Level: $28,805.

Norman W. Heimstra, Ph.D.
Professor of Psychology
Director, Human Factors Laboratory
University of South Dakota
Vermillion, South Dakota 57069

Behavioral Effects on Nonsmokers of Exposure to Smoking.

There is a growing trend for legislative bodies and other groups to legally prevent smoking in a variety of different situations. This has come about largely because of increased vocalization on the part of nonsmokers. The aim of this investigation is to determine the behavioral effects on nonsmokers when they are exposed to situations where smoking takes place. Particular attention will be paid to the variables (personal, situational, task) which may lead to a state of annoyance in the nonsmokers, to possible deterimental effects on performance of nonsmokers exposed to smoking, and to changes in social behavior of nonsmokers in these conditions.

Activation Date: September 1, 1976

Current Grant Level: $18,080.

William J. Jusko, Ph.D.
Associate Professor of Pharmaceutics
Director, Clinical Pharmacokinetics Laboratory
Millard Fillmore Hospital
Three Gates Circle
Buffalo, New York 14209

Effect of Smoking and Its Cessation on Drug Disposition.

Cigarette smokers appear to differ from nonsmokers in their responses to pharmacologic agents and in rates of drug disposition. This may be due partly to induction of drug metabolizing enzymes. These expectations indicate the need for examination of the pharmacokinetics of some clinically important drugs in relation to smoking habit. Since smokers may also differ from nonsmokers in other complicating respects (coffee and alcohol ingestion, drug history, body fat and protein composition, basal metabolic rate, etc.), a proper evaluation of the effects of smoking requires statistical examination of these factors in testing smokers and nonsmokers and use of subjects as their own controls (cessation of smoking).

The role and effect of cigarette smoking on the pharmacokinetics of selected drugs will be examined in healthy adult subjects by the following studies related to some clinically-important drugs:

1. Determination of the distribution and elimination kinetics of theophylline, phenytoin and propranolol in nonsmokers and heavy smokers.

2. Examination of the long-term effects of cessation of smoking on the disposition kinetics of theophylline and on the urinary excretion of D-glucaric acid, an index of hepatic enzyme induction.

3. Assessment of the role of multiple factors which may account for or contribute to possible differences in drug disposition between smokers and nonsmokers.

Activation Date: July 1, 1976

Current Grant Level: $21,175.

Edward L. Klaiber, M.D.
Senior Scientist
The Worcester Foundation for
Experimental Biology, Inc.
222 Maple Avenue
Shrewsbury, Massachusetts 01545

Studies of a Gonadal and Central Nervous System Syndrome that Differentiates Smokers from Nonsmokers.

The objective of this research is to define whether there are physiological and psychological parameters which differentiate smokers from nonsmokers in a young adult male population. It is hypothesized that any differences may be in part due to the effect of smoking, and in part to constitutional factors which precede smoking but which predispose an individual toward smoking. The investigators' findings at this point indicate that smokers have a central nervous system adrenergic dysfunction which may be secondary to a relatively decreased production of testosterone. Smokers also have an increased incidence of testicular varicoceles, (38% vs. 11% in nonsmokers). This abnormality may affect gonadal function and impair hormone production. In addition, significantly more smokers than nonsmokers are born in winter months (October thru March) and fail to exhibit laterality as measured by handedness and biceps circumference. These latter differences may represent antecedent factors related to smoking.

The specific aims of the study are:

(1) To study 500 young adult males (250 smokers, 250 nonsmokers) between the ages of 18 and 25, with respect to (a) incidence of testicular varicoceles; (b) month of birth, and (c) handedness and physical laterality as specified by biceps, forearm, calf and thigh circumferences.

(2) To study the dynamics of testosterone production and clearance rates in some of these same subjects.

Data are being collected now.

Activation Date: June 1, 1976

Current Grant Level: $30,000.

Gerald E. McClearn, Ph.D.
Director, Institute for Behavioral Genetics
Department of Psychology
School of Pharmacy
University of Colorado
Boulder, Colorado 80309

Heredity and Tobacco-Related Behavior in the Mouse.

Although a substantial literature has accumulated concerning the relevance of heredity to a variety of behaviors related to drugs, relatively little research has been done on genetics and tobacco use. A few published studies of human twins' smoking behavior and of differential behavioral response of inbred mouse strains to administration of nicotine strongly suggest that there are important hereditary influences on tobacco-related behavior, but the total evidence can only be regarded as promissory. The general objective of the proposed research is to further knowledge in this area, using the mouse as an experimental organism.

Evidence will be sought for genetic influence on individual differences in (1) acceptance or preference for nicotine and other tobacco components, and (2) sensitivity to the effects of administered nicotine and other tobacco components on locomotor activity, gregarious (grouping) behavior, aggression, sexual behavior, and learning and memory. Related to sexual behavior will be research on reproductive performance.

Biochemical and neurophysiological mechanisms underlying genetically-influenced differences in tobacco-related behavior will be investigated. Such differences may be related to variations in tolerance, dependence, metabolic parameters, and central nervous system sensitivity (as assessed by convulsion thresholds, cortical desynchronization, hippocampal theta rhythms, sensory evoked potentials, and single-cell responses).

Activation Date: April 1, 1976

Current Grant Level: $164,755.

Stephen M. Ayres, M.D.
St. Vincent's Hospital and Medical Center of New York
153 W. 11th Street
New York, New York 10011

Effects of Tobacco Smoking and Community Air Pollution on Myocardial Metabolism. Grant #668R2

Studies are being conducted into the distribution of carboxyhemoglobin (COHB) in the general population and the effects of carbon monoxide (CO) on the cardiovascular system. A sensitive alveolar sampling technique utilizing gas chromatography has been developed and used to determine the COHB distribution in a sample of 3000 subjects. These data are correlated with the responses of the subjects on a questionaire describing smoking history and other contacts with CO. Significant numbers of smokers were found with COHB saturations above 3.5%. Tunnel workers have been tested and found to have excessive levels of COHB. These workers are exposed to CO concentrations of up to 300 ppm. The synergistic effects between CO and nicotine are being studied in canine preparations. Preliminary evaluation indicates that the effects of 5% CO and nicotine on the myocardium are similar to previously reported work while administration of 0.1% had a significantly less dramatic effect. Studies with human subjects showed a decrease in arterial oxygen tensions following carbon monoxide breathing and increased minute ventilation, oxygen consumption, and cardiac output. Many nonsmokers with coronary disease have COHB saturations approaching that of smokers. Preliminary analysis has not revealed significant differences in either coronary anatomy or myocardial metabolism of smokers compared to nonsmokers, but we have not been impressed with the experimental data presented and are continuing a serial evaluation of all patients referred to coronary arteriography who are found to have coronary artery disease.

Current Grant Level: $30,382.

Samuel Bellet, M.D.
Philadelphia General Hospital
34th Street & Civic Center Boulevard
Philadelphia, Pennsylvania 19104

Effect of Nicotine on Various Parameters of Cardiovascular Function - Effect of Smoking on Myocardial Fibrillation Threshold. Grant #607-C

Previous studies have shown that nicotine administration results in a significant increase in catecholamine release. As catecholamines are known to increase the irritability of the myocardium, it was felt that the effect of smoking on the fibrillation threshold would be of interest. Because of its action on catecholamine release, one would anticipate that cigarette smoking might increase myocardial irritability and, therefore, decrease the fibrillation threshold.

The purpose of this study is to determine the effect of cigarette smoke inhalation on the ventricular fibrillation threshold (VFT) in dogs. The VFT was determined in 18 normal dogs by closed chest method using external thoracic electrodes. Cigarette smoke inhalation was accomplished by attaching three lit cigarettes in succession to one end of a "Y" rubber tube connected to the air input of the respirator. The VFT decreased from a control value of 1.02 wsec. to 0.79 wsec. at 15 minutes ($p<0.01$), 0.67 wsec. at 30 minutes ($p<0.02$), 0.59 wsec. at 45 minutes ($p<0.01$), 0.65 wsec. at 60 minutes ($p<0.01$), and 0.67 wsec. at 90 minutes after cigarette smoke inhalation ($p<0.01$). In the control experiments no significant decrease in VFT was observed. These results indicate that cigarette smoke inhalation results in a significant decrease of the VFT in dogs. These findings are of interest in view of the increased incidence of sudden death observed in coronary subjects who are heavy cigarette smokers. Present studies are being done in dogs with experimental myocardial infarction in various stages.

Current Grant Level: $17,870.

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Facilitation of Vasoconstriction by Nicotine and Related Agents.

Grant #690

Despite the considerable scope of current research on the cardiovascular actions of nicotine, our recent observation suggests that a potentially important action of nicotine may have been overlooked.

In our studies using the isolated pulmonary artery of the rabbit, nicotine was found to increase the vasoconstrictor response to sympathetic postganglionic nerve stimulation. This effect took place with nicotine concentrations ($0.5\text{-}1.5 \times 10^{-6}$M) too low to elicit any significant vasoconstriction in the absence of sympathetic nerve stimulation, and should hence be termed "facilitation." Higher concentrations of nicotine ($1.5\text{-}15 \times 10^{-5}$M) elicited vasoconstriction as expected, and this resulted from the releasing effect of nicotine on norepinephrine (NE) contained in the vasoconstrictor nerves. However, these high concentrations are obviously less likely to be attained in vivo following tobacco smoking. In this sense the vasoconstrictor effect of nicotine is not so relevant as its facilitatory effect associated with lower concentrations. The role of this effect apparently has not been fully understood, and more knowledge is needed to allow more rational control of the cardiovascular effects of nicotine and other facilitators.

The aim of this project is first, to characterize and quantitate the facilitatory effect of nicotine on the transmission between vasoconstrictor nerve and vascular smooth muscle. It is then planned to elucidate the mechanism of this nicotinic action in light of the mechanisms of action of other facilitators. Finally, it is hoped to explore the availability of specific agents which may be useful in controlling the facilitatory effect of nicotine.

Current Grant Level: $14,416.

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Saint Louis University School of Medicine
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Central Action in Nicotine (Chronic Administration).

It is generally agreed that nicotine plays an important role in the tobacco smoking habit. It has been suggested that nicotine might produce some of its action in the central nervous system by the release of norepinephrine or other amines from the stores in the brain. However, no definite changes in the catecholamine levels in the brain were observed after acute or chronic administration of nicotine. In contrast, the chronic treatment with nicotine markedly increased the turnover rate of norepinephrine. The present study is aimed to determine 1) the step at which norepinephrine synthesis is increased and the part of the brain in which the turnover rate is increased most by chronic administration of nicotine; 2) the rate of utilization of ^{3}H-norepinephrine in brain of these experimental rats; 3) the time at which the turnover rate of norepinephrine begins to increase after repeated administration of nicotine, the time at which it reaches its maximum rate, and the time when it returns to its normal levels following withdrawal of nicotine; 4) if there is alteration in the turnover of acetylcholine and other amines (e.g., serotonin) by chronic administration of nicotine.

Thus, electrophysiological, behavioral and biochemical approaches will be used in this study of effect of nicotine and tobacco smoking upon the central nervous system. It is hoped that this study will aid in the elucidation of the central mechanism of nicotine.

Activation Date: February 1, 1971

Current Grant Level: $24,115.

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Mechanisms of the Action of Carbon Monoxide on Atherosclerosis.

The objective of this work is a study of the mechanisms of carbon monoxide on cholesterol transfer in perfused coronary arteries.

The study may be divided into two approaches: (1) physico-chemical mechanisms; and (2) the study of hemodynamic causes of increased cholesterol transfer.

The question to be examined is, "Can increased permeability be artificially produced?" These studies will be carried out using collagenase, an enxyme which has been shown by Jaffe and associates to lead to digestion of portions of the vascular wall, the degree of change depending on the time to which these vessels are exposed to the enzyme.

Activation Date: July 1, 1974

Current Grant Level: $45,293.

Bruce F. Cameron, M.D., Ph.D.
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Spectrophotometric Assay of Carbon Monoxide and Nitric Oxide Hemoglobin.

Grant #661R2

A continuous recording polarographic apparatus for measuring hemoglobin oxygen dissociation is being set up, for examination of the effect of carbon monoxide and nitric oxide on oxygenation.

Primary population groups for study include homozygous and heterozygous examples of hemoglobin S and hemoglobin C, anemia, pregnancy, and ageing.

Human myoglobin has been isolated and crystallized, and its physiologic properties are under study. This is especially concerned with the linked interaction between oxygenation or carbon monoxide binding and an unidentified low MW ligand normally present in muscle extracts.

Current Grant Level: $16,192.

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Effects of Tobacco Smoke and Nicotine on Coronary Collateral Blood Flow.

It is the investigators' intent to determine the effects of (1) tobacco smoke and (2) nicotine, on coronary collateral blood flow following acute or chronic occlusion of a coronary artery. In addition, they will determine the effects of these agents on the distribution of cardiac output.

Following either acute or chronic occlusion of the anterior descending coronary artery, regional blood flow will be measured by tissue uptake of radioactive microspheres in anesthetized open-chest dogs. Differently labeled microspheres will be used for control and experimentatal flow measurements in each animal.

Activation Date: July 1, 1974

Current Grant Level: $15,290.

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The Influences of Smoking and Nicotine on Circulation in the Lower Limb.

The proposed work is designed to assess the influences of smoking and of nicotine on circulation in the human leg and in the dog leg. Parameters to be assessed will be skin temperature, arterial and venous blood gases, arteriovenous anastomotic blood flow, and arteriovenous oxygen differences, muscle blood flow, and skin blood flow. Human subjects will smoke cigarettes or receive a nicotine injection. Animal experiments will be done, using fractions of cigarette smoke or nicotine injections.

Activation Date: January 1, 1971

Current Grant Level: $12,542.

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Effects of Nicotine on Interactions of Platelets and Endothelial Cells.

The effect of nicotine upon the interaction of platelets in plasma or of isolated platelets with endothelial cells will be investigated. In addition, the effect of nicotine on platelet-platelet interaction and upon endothelial cell-endothelial cell interaction will be investigated. Aggregations of platelets is an initial step in thrombus formation. Adhesion of platelets to the endothelial cells could constitute one of the earliest steps in thrombus formation. Interaction of platelet aggregates with endothelial cells could produce alterations in endothelium leading to promotion of thrombosis. Investigation of the interactions of endothelial cells and platelets should lead to a better understanding of the roles of these cell types in thrombosis. Studies of the influence of nicotine upon these cellular interactions should clarify understanding of possible effects of smoking on thrombosis.

Platelets will be obtained from healthy donors. Endothelial cells will be obtained from the umbilical cord vein and will be used directly or after growth in tissue culture. Platelets will be mixed with endothelial cells in an aggregometer in the presence and absence of varying concentrations of nicotine. Cellular interactions will be followed by photometry, phase-contrast microscopy and electron microscopy. Release of substances from platelets or endothelial cells which promote or inhibit cellular interactions will be sought.

Activation Date: July 1, 1974

Current Grant Level: $30,000.

Una Smith, Ph.D.
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The Role of Endothelial and Epithelial Cells in Non-Ventilatory Functions of the Lungs.

The principal aim of this study is to examine the role of pulmonary endothelial and alveolar epithelial cells in the metabolism and removal of vasoactive substances in the venous circulation. The intent is to continue testing the hypothesis that pulmonary endothelial cells are actively engaged in the metabolism of circulating adenine nucleotides, bradykinin and angiotensin I. However, evidence at hand indicates that other circulating substances, e.g., the prostaglandins, may not be metabolized by endothelial cells. Therefore, these studies will be extended to include an examination of alveolar epithelial cells and their possible role in the metabolism of prostaglandins and in the production of surfactant.

Specifically, the fine structure of endothelial and alveolar type I and II cells, interactions between these cells, effects of hormones, nicotine and respiratory gases on fine structure, cytochemical reactivities, and the ability of these cells to carry out specific hydrolase reaction will be studied. Methods will include freeze-etching and autoradiography at the level of electron microscopy.

As the normal structures and functions of the endothelial and epithelial cells are clarified, plans are to examine the effects of tobacco smoke on specific hydrolytic reactions of the endothelial cells and on the formation and maturation of lamellate bodies of alveolar type II epithelial cells.

Activation Date: October 1, 1972

Current Grant Level: $32,908.

Richard L. Naeye, M.D.
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Smoking and the Pulmonary Blood Vessels: A Quantitative, Morphologic Study.

Grant #698R1

1. There has been a report that cigarette smoking induces a "fibrous" thickening of small pulmonary and systemic arteries and arterioles. This report is based on a subjective analysis. We are applying quantitative, histologic methods to determine if such vessels have a different structure in smokers and nonsmokers.

2. Coal workers often develop cor pulmonale. We are undertaking a quantitative, morphologic analysis of the lung in an attempt to determine which abnormalities might be responsible for the cor pulmonale. Smoking history will be considered in the final analysis of the data.

Current Grant Level: $27,537.

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The Action of Nicotine on the Adrenal Gland.

The physiological actions of adrenal gland hormones are observed in most tissues and are vital for the normal functioning of many organs. The effects of medullary catecholamine release, for example, include hyperglycemia, hyperlipemia, peripheral vasoconstriction, and an increase in the work of the heart. Enhanced corticosteroid release produces marked changes in water and electrolyte balance and in carbohydrate, fat, and protein metabolism. Thus, any agent which can affect the production and release of adrenal hormones may have marked effects on bodily functions. Nicotine is an agent which can enhance both catecholamine and corticosteroid release from human and animal adrenal glands, so that basic knowledge of the effects of nicotine on adrenal activity is of considerable importance in order to help elucidate hormone secretory mechanisms. Moreover, such information may allow a better assessment of the effects of tobacco smoking on the adrenal cortex and medulla.

The prime thrust of this investigation will be to systematically study the effects of nicotine on both catecholamine and corticosteroid release from the cat adrenal gland perfused in situ, in order to elucidate the mechanisms by which it enhances adrenal activity. Not only will hormone production and release be assayed under various experimental conditions, but other cellular processes, which are presumed to be related to the physiological response will also be investigated, as for example, the possible relationship of adrenal cyclic AMP levels and radiocalcium fluxes to hormone release.

Activation Date: July 1, 1974

Current Grant Level: $16,675.

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Constitutional Studies Relative to Smoking and Coronary Heart Disease.

Raw data are available to the principal investigator from a number of sources with respect to a number of studies. In order to expedite the preparation of manuscripts, professional personnel are necessary for certain aspects of the analysis. Raw data are available as to manifold differences between smokers and nonsmokers, between nonsmokers and ex-smokers, and between pipe and cigar smokers from Kaiser-Permanente, Framingham and/or Veterans Outpatient Clinic data bank. Also from the same source manifold data (laboratory, morphological, physiological, medical, personality, educational, etc.) are waiting for analysis. From the various published studies, data are available as to the CHD risk of elderly men and women (ages 65-84), which must be assembled in proper form, appropriate tables prepared, age-standardized death rates computed, various literature references assembled. With this preparation, manuscripts can be written. The subject of pipe and cigar smoking in relation to risk of disease and death must also be assembled. Other studies must also be prepared.

Many of these preparatory activities can be performed simultaneously by different persons deemed competent to work at these projects.

Activation Date: July 1, 1974

Current Grant Level: $16,250.

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Variables Affecting the Cardiovascular Responses to Chronic Smoking.

Although several epidemiologic and pathologic studies have suggested an increased incidence of cardiovascular disease in the smoking population, a direct causal relationship between chronic smoking and cardiac disease has not been established. This project will study whether the heart is affected by chronic smoking of cigarettes in the experimental animal under control conditions at a usage level that is common in man. The effects of smoking the equivalent of one or two packs of cigarettes/day in man is to be determined in purebred beagles with chronic tracheostomies, using litter mate controls. The investigators' preliminary studies in the beagle have suggested that cardiovascular abnormalities may be produced with relatively large cigarette exposure. They wish to determine the threshold for this response and the influence of dietary variables.

Smoking animals receiving high lipid diet will be matched with appropriate controls to determine if plasma lipid levels may affect the cardiac response to smoking. Since smoking is often combined with moderate to heavy ethanol intake in man, an agent which can be associated with cardiac disease in humans and experimental animals, the researchers propose to examine its interaction with cigarette inhalation and whether quantitative or qualitative differences are observed when compared to controls. They are particularly interested in evaluating alterations of blood coagulation as a potential factor in diminishing blood flow in the coronary microcirculation and to altered myocardial function. Their early studies in the purebred beagles indicate that a hypercoagulable state is induced in the smoking dogs compared to their litter mate controls during the initial 12-18 months. The relationship of the development of hypertension to the turnover of ^{125}I-labeled fibrinogen and deposition of the protein in the microcirculation of the kidney and myocardium is to be assessed. The investigators propose to determine whether progression of this abnormality occurs with time and the interrelations of altered myocardial function, metabolism and morphology.

As an important correlative study, the morphologic status of the lungs and pulmonary vasculature in these animals will be evaluated by Dr. Sheldon Sommers, professor of pathology at Lenox Hill Hospital, New York City.

Activation Date: January 1, 1975

Current Grant Level: $47,540.

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Endocrine Functions of the Lungs.

There are two main aims of this research project: the first is to obtain direct evidence on the precise subcellular site of pulmonary angiotensin converting enzyme and to relate these data to understanding of the nonventilatory functions of the lung. The second aim is to develop rapid, simple and sensitive assays of pulmonary angiotensin converting enzyme to facilitate future studies of the effects of physiologic changes and environmental influences (e.g., tobacco smoke) on the ability of the lungs to convert angiotensin I to its potent lower homolog, angiotensin II.

It is now well recognized that the lungs process the circulating vaso-active polypeptides, bradykinin and angiotensin I. Previously, these investigators have postulated that the enzymes responsible for the metabolism of these polypeptides are situated on the luminal surface of pulmonary capillary endothelial cells. Studies from their laboratory over the past six years, as well as from others, have supported the hypothesis but have not proved it. Within the last year, it has been shown that the enzyme that activates angiotensin I (by conversion to angiotensin II) is also responsible for the inactivation of bradykinin. These investigators have raised antibodies to this enzyme, and now propose to use the antibodies labeled for immunocytochemistry to provide a definitive test of their hypothesis.

In addition to testing the hypothesis described above, they would like to undertake a technical program to develop inexpensive, radioactive substrates which will lend themselves to the rapid, simple and sensitive measurement of the turnover rate of angiotensin converting enzyme. Such substrates will be of immediate use in investigations of the ways in which the lungs can modulate their "endocrine" functions in response to physiological stimuli and to inhalants such as tobacco smoke.

Activation Date: January 1, 1975

Current Grant Level: $37,902.

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Study of Sensitivity of Vascular Tissue to Nicotine. Grant #681R2

In a study of the relationship between nicotine's action and the catecholamine metabolic inhibitor on the vascular smooth muscle, rabbit spiral aortic strips were used. After treatment with a-m-p-tyrosine, which inhibits tyrosine hydroxylase, nicotine-induced contractions were remarkably reduced by 30% of the untreated preparations. Also, metaraminol (m-hydroxynorepinephrine) releases and replaces norepinephrine at the norepinephrine storage site. It inhibits nicotine-induced contractions by 40% of the untreated control preparation. Furthermore, reserpine treatment abolished the response to the nicotine, but the guanethidine treatment reduced such a response only slightly.

Similar results were observed on the tyramine-induced contraction. However, inhibitory action of the above agents are much less than that of nicotine's. The decreased response to nicotine after treatment with metaraminol and a-m-p-tyrosine was recovered by dopamine-incubation, but not by exogenous norepinephrine-incubation.

Dissulfiram (tetra-ethylthiuran), which inhibits dopamine-b-hydroxlase, has no effect on nicotine induced contractions. It may be explained that the rabbit processes norepinephrine by a different possible biosynthetic pathway. It could bypass dopamine-b-hydroxylase and follow the octopamine CFE pathway.

The results may suggest that nicotine contractions are mediated by catecholamine release from the storage site and that exogenous norepinephrine may not be available for nicotine-induced contraction.

Current Grant Level: $25,497.

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A: The Acute Effect of Smoking upon Regional Cerebral Blood Flow in Smokers and Nonsmokers. Grant #752

B: The Effect of Some Physiological Stimuli upon Cerebral Blood Flow in Smokers and Nonsmokers.

By means of the intracarotid ^{133}Xe injection method for determination of regional cerebral blood flow this is measured simultaneously in 35 areas of the brain. The questions to be answered are:

1. Does actual smoking influence cerebral blood flow globally or regionally?

2. Is the oxidative metabolism determined by flow and a-v oxygen difference influenced by smoking?

3. Is the possible effect of actual smoking different in smokers and nonsmokers?

4. Is the reactivity of cerebral vessels upon controlled changes in BP and in $ApCO_2$ different in a group of smokers and in a group of non-smokers comparable from other points of view?

Current Grant Level: $8,100.

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Role of Lecithin: Cholesterol Acyltransferase (LCAT) in Cholesterol Metabolism in Health, Disease and During Smoking.

The objective of this project is to increase fundamental information concerning the metabolism of blood cholesterol which occupies the center of the predominant theory of the pathogenesis of atherosclerosis. Specifically, the investigator's objective is to determine why cholesterol exists in two forms in the blood -- lipoprotein-free cholesterol and lipoprotein cholesterol esters -- and the physiologic function of each type of cholesterol.

The experimental approach will be to purify and develop an antibody to LCAT, the enzyme responsible for esterifying blood cholesterol. The purification scheme involves preparative ultracentrifugation, affinity chromatography and column chromatography to obtain a preparation which is not only pure but stable. The chemical properties of the enzyme will then be analyzed. The scheme for production of antibody to LCAT consists of immunizing rabbits to a pH 9 preparation and differentially eluting the one to three components present in this highly concentrated preparation of LCAT.

The investigators now have a preparation which is concentrated at least 10,000-fold, stable for days and which contains one major and one minor component, as judged by disc-gel electrophoresis. They are also determining the activity of the enzyme in the processing of specific apoproteins and testing their antibodies.

Activation Date: July 1, 1974

Current Grant Level: $65,000.

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Cigarette Smoking Patterns over Time: Profiles of Former Smokers Compared with Continuing Smokers and Nonsmokers. Grant #610-B

The study population consists of 1130 white males who entered the study as medical students at a median age of 22 years. At entry, 363 were nonsmokers and 517 were cigarette smokers of some type; the remainder smoked pipes, cigars, or a combination thereof, or had smoked cigarettes briefly and then stopped. Subjects entered the study between 1947 and 1961 in classes graduating from medical school in 1948 through 1964. In 1968, there were 312 continuing cigarette smokers, 543 non-cigarette smokers, and 272 subjects who had stopped smoking cigarettes. Using this population, it is proposed to:

(a) compare the characteristics in youth of physicians who have stopped smoking cigarettes with those who are continuing to smoke and those who are nonsmokers.

(b) relate the smoking habit patterns to several parameters, notably hypertension and coronary disease.

Current Grant Level: $25,955.

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Peter Brent Brigham Hospital
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Nicotine-Induced Reflex Coronary Vasodilation.

The goal of this study is to delineate the mechanism by which intravenous nicotine produces coronary vasodilation. Specifically, it is proposed to: (1) demonstrate that the nicotine-induced coronary vasodilation is reflex in nature and (2) establish the afferent and efferent pathways involved in this response. It is know that nicotine stimulates chemoreceptors, which in turn elicit hyperventilation. It is proposed that the resultant hyperventilation, through stimulation of the lung inflation reflex, is responsible for the coronary vasodilation.

Several approaches will be made to this problem. The extent to which intravenous nicotine increases coronary blood flow and reduces coronary vascular resistance will be determined. Whether the nicotine-induced coronary vasodilation is primary or secondary to changes in myocardial O_2 requirements will be examined by comparing effects of intravenous and intracarotid nicotine on coronary dynamics (1) when the heart is in spontaneous rhythm, (2) with heart rate controlled by pacing, and (3) after inotropic effects are blocked with propranol, 1 mg/kg. In addition, arterial and coronary sinus oxygen will be measured to determine if coronary A-VO_2 difference narrows.

The efferent autonomic pathways involved in the changes in heart rate, total peripheral resistance and resistances in the coronary, mesenteric, renal and iliac beds will be determined by selective and combined blockades of beta adrenergic receptors with propranolol, 1 mg/kg, alpha adrenergic receptors with phentolamine, 1 mg/kg, and cholinergic receptors with atropine, 0.2 mg/kg.

The extent to which nicotine-induced circulatory changes, and the induced coronary dilatation in particular, are due to nicotine's chemoreceptor stimulating action will be determined by injecting nicotine through catheters previously implanted in the common carotid artery just proximal to the carotid sinus.

The extent to which hyperventilation, through stimulation of pulmonary stretch receptors, resulting from nicotine's stimulation of chemoreceptors, regulates the circulation and specifically causes coronary dilation will also be examined by hyperinflating the lungs in conscious animals. The effects of pulmonary hyperinflation on heart rate, arterial pressure, myocardial contractility, cardiac output, total peripheral resistance and blood flow and resistance in the coronary, mesenteric, renal and iliac beds will be examined.

Activation Date: July 1, 1974

Current Grant Level: $20,000.

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Pennsylvania State University
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Pharmacokinetics of Nicotine in Naive and Habituated Cigarette, Cigar and Pipe Smokers.

The objectives of this project are (1) to describe quantitatively changes in nicotine blood concentrations of habituated and naive smokers of cigarettes, cigars and pipes after smoking, (2) to define precisely the range of variations in nicotine plasma concentrations in these different groups of subjects after smoking, and (3) to determine whether chronic nicotine administration causes induction of the hepatic microsomal drug-metabolizing enzymes responsible for the biotransformation of nicotine.

Using the radioimmunoassay, new naive and new habituated cigarette smokers, cigar smokers and pipe smokers will be observed to determine the range of variation in plasma nicotine concentration that occurs during inhalation of set numbers of puffs of cigarette, cigar and pipe smoke, respectively, at each of the time points selected.

The radioimmunoassay to detect nanogram quantities of nicotine in human plasma, which these investigators have developed over the past three years, will permit them to process the large number of samples required for pharmacokinetic studies. This makes possible establishment of correlations between various effects of nicotine and plasma nicotine concentrations, and a correspondingly better understanding of the epidemiological, physiological, psychological and genetic bases of tobacco usage in the general population.

Activation Date: December 1, 1974

Current Grant Level: $26,230.

CHRON. RESP. DIS.

1005075501

Animal Lung Stdy

1005075502

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Influence of Cigarette Smoke on Pulmonary Emphysema and Bronchospasm.

The objective of this project is to examine the significance of cadmium contained in cigarettes which has been implicated as the probable cause of pulmonary emphysema and bronchitis occurring in cigarette smokers.

Mice and rats will be exposed to cigarette smoke with varying concentrations of cadmium. The lungs will be examined functionally and histologically.

Various strains of mice are being examined to identify those that are susceptible to emphysema or bronchitis.

Activation Date: July 1, 1974

Current Grant Level: $38,064.

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Cigarette Smoke Effects on Certain Aspects of Rat Lung Metabolism.

These studies are intended to accomplish the following specific research aims:

(1) to determine the effects of long-term cigarette smoking on rat lung enzymes related to the pentose shunt and to glutathione metabolism; on rat lung lysosomal enzymes; on rat lung alveolar macrophages; and on rat antibacterial defense mechanisms.

(2) to determine the effects of long-term cigarette smoking minus particulates on the above mentioned lung biochemical and antimicrobial parameters.

(3) to determine the interacting effects of long-term cigarette smoking and oxidant pollutant (ozone) exposure on the above mentioned lung biochemical and antimicrobial parameters.

Observations of potential NADPH-related antioxidant defense system augmentation in response to cigarette smoke exposure will also be followed up (particularly with regard to the interacting effects of oxidant pollutants) and the participation of the superoxide dismutase antioxidant defense systems in this overall reaction scheme will be studied. It is also intended during the second year to gather preliminary information concerning the effects of cigarette smoke on lung fibrogenesis, on lung nucleotide synthesis rates, and on lung prostaglandin synthesis and degradation.

Activation Date: January 1, 1975

Current Grant Level: $43,400.

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The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function.

Proposed studies are a comprehensive evaluation of the influence of cigarette smoke on pulmonary defense against inhaled bacteria. The specific parameters of pulmonary defense to be assessed include total lung clearance of inhaled bacteria, alveolar macrophage activity, and mucociliary function in the normal situation and during the inhalation of cigarette smoke.

The procedure of this research depends on four major techniques: (a) depositing predictable numbers of aerosolized bacteria in the trachea and lungs of animals; (b) harvesting alveolar macrophages; (c) quantitating the antibacterial activity of alveolar macrophages; and (d) producing reproducible concentrations of puffed cigarette smoke.

Experiments are proposed which permit an evaluation of the influence of extended exposure to cigarette smoke on the following parameters of alveolar macrophage activity: (a) oxygen uptake; (b) glucose metabolism; and (c) hydrolytic enzyme activity.

Activation Date: July 1, 1973

Current Grant Level: $22,944.

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The Effect of Smoking on the "Small Airways."

The aim of this project is to determine the acute and chronic effect of tobacco smoke on "small airway" resistance.

The investigators are measuring the flow-volume-pressure relationship in the respiratory system before and after smoking one cigarette and as a function of smoking habits. Measurements include maximum expiratory flow-volume curves, closing volume, frequency dependence of dynamic compliance, nitrogen washout at different frequencies of breathing, etc.

Dose dependence of "small airway" function on cigarette smoke was assessed. The acute effect of cigarette smoke on flow volume loops using gases of different density has been assessed. A large number of presently used tests for assessment of "small airway" function have been administered to subjects with known smoking history and their comparative values in identifying smokers were determined.

Activation Date: January 1, 1975

Current Grant Level: $28,265.

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The Role of the Macrophage in the Immune Response: Effect of Tobacco Products on Macrophage Function.

It has been suggested that the immune system plays a role in the regulation of expression of malignant cell clones. Macrophages participate as accessory cells in both the afferent and efferent arms of the immune response. Several studies have suggested that interference with macrophage function results in an altered immune response. Further, it has been shown that smoke and/or tobacco products result in alterations of normal phagocytic functions of macrophages, however there is a paucity of information regarding the effect of tobacco on immune function. The objective of this study is to determine the effect of cigarette smoke on the immune response of mice after chronic and acute exposure.

Initial experiments will be carried out on the in vivo effects of cigarette smoke on the immune response. Mice will be exposed to Kentucky Standard (1A1 and 1R1) Cigarette smoke on a Prototype Walton Mark II Horizontal Smoke Exposure Machine for varying periods of time. The following parameters will be investigated: acute vs. chronic exposure, time of exposure in relation to time of antigen stimulation, primary vs. secondary immune response, recovery time from smoke exposure, dose duration and frequency of smoke exposure, and possibly animal strain differences. These experiments will then be carried out in vitro by exposing culture macrophages to smoke and assessing their immune function.

The investigators have already described the system to study the in vitro anamnestic response of a dispersed lymphoid cell population. They have systems in routine operation for the evaluation of antibody-forming cells, and they have established methodology to separate lymphoid cell populations into functional types. The researchers have established a participatory role of macrophages in the anamnestic response to KLH. Animals are currently being introduced into the protocols described above.

Activation Date: October 1, 1974

Current Grant Level: $33,000.

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Immune Mechanisms of Mucous Membranes.

The objective of this study is to learn whether or not there are abnormalities of the local secretory immune system in patients with chronic respiratory disease.

The approach used will be to evaluate autopsy and biopsy specimens by immunofluorescence of immunoglobulin H chains and secretory component.

Current plans are to begin these evaluations.

Activation Date: January 1, 1975

Current Grant Level: $34,752.

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The Lymphatics of the Lung: Their Role in Fluid Transport and Clearance of Airborne Particulate Matter in Normal and Experimental Conditions and in Various Lung Diseases.

The morphology of the pulmonary lymphatics and their role in pulmonary fluid transport and airborne particulate matter and in various lung diseases will be investigated, using light optical and electron microscopical methods.

Activation Date: January 1, 1975

Current Grant Level: $27,016.

Clayton G. Loosli, Ph.D., M.D.
Hastings Professor of Medicine & Pathology
School of Medicine
University of Southern California
2025 Zonal Avenue
Los Angeles, California 90033

Effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of Mice.

This is a continuation study of the effects of fresh cigarette smoke inhalation on the respiratory tracts of SPF mice, employing Mark II Walton Horizontal Smoke Exposure Machines. The low nicotine reference cigarette (1A1), prepared by the Tobacco and Health Research Institute of the University of Kentucky, is used.

Groups of C57Bl/6J, C57L/J and SWR/J mice are being exposed daily to the smoke from 1, 2, 3, and 4 cigarettes. Each smoke exposure comprises a 2-second puff, (35 ml) introduced into a chamber into which the noses of 20 mice protrude. The smoke by volume in the chamber is approximately 8.5%. Exposure to the smoke is 18 seconds after which the chamber is purged with fresh air. The smoke exposure cycle is repeated at one-minute intervals for eight puffs. Appropriate groups of mice serve as shelf and sham controls.

After one-year exposure to the smoke, the mice will be sacrificed and comparative studies of the lungs of smoke-exposed and nonexposed mice will be made. Quantitative and qualitative differences in lung reactions, in relation to the total numbers of cigarette smoke exposures, will be made and compared to changes seen in the lungs of control mice.

Activation Date: October 1, 1974

Current Grant Level: $60,000.

Donald Massaro, M.D.
George Washington University School of Medicine
Washington, D. C.

Protein Synthesis and Secretion by Tracheal Mucosa. Grant #645R2

Although cells derived by lavage of animal lungs have been studied in great detail with regard to their phagocytic, enzymatic, and certain other biochemical properties, little information exists regarding protein synthesis in these cells. We are studying the synthesis, transport, and release of proteins by these cells.

Current Grant Level: $27,107.

Daniel B. Rifkin, Ph.D.
Assistant Professor of Chemical Biology
The Rockefeller University
New York, New York 10021

Proteases Produced by Mammalian Lung Tissue.

The aim of this project is the characterization of a low molecular weight protease produced by human embryonic lung cells in vitro. This protease is a potent activator of serum proenzymes and may be involved in certain pathological conditions of the human lung, namely respiratory distress syndrome (RDS) and chronic emphysema. The proteases from human and mouse lung cells grown in culture will be purified and characterized as to their physical properties, mode of action, substrate specificity, and ability to activate proenzymes such as plasminogen procollagenase and proelastase. Antibodies will be made to the purified proteins. These antibodies will be used to determine at what stage in the embryonic development of the mammalian lung this enzyme appears and if it continues to be produced after parturition. Fluorescein-labeled antibody will be employed as an immunofluorescent probe to test if other cells in preparations of fixed lung tissue are producing this enzyme. Finally, experiments will be designed to ascertain whether the production of the lung enzyme can be influenced by drugs such as corticosteroids.

It is hoped that this analysis will provide information necessary to determine if this protease may be involved in the respiratory disease and, if so, how one may develop therapeutic approaches to these conditions.

Activation Date: January 1, 1975

Current Grant Level: $36,000.

James Travis, Ph.D.
Associate Professor of Biochemistry
University of Georgia
Athens, Georgia 30602

Biochemistry of Chronic Obstructive Lung Disease.

This project involves the study of the properties and function of both alpha-1-antitrypsin and leukocytic elastase(s) from human blood. The study is designed to test whether lack of alpha-1-antitrypsin can cause emphysema by allowing leukocytic elastase(s) to digest basement membrane in the lung.

Both proteins have now been isolated in a relatively pure form and antibodies are being prepared to each. The experimenters will fluorescent-label each and by histological section attempt to deposit each antibody on lung taken from individuals who are homozygous deficient for the alpha-1-antitrypsin. In theory, the antibody to elastase(s) should be deposited while that to the alpha-1-antitrypsin should not.

They are also interested in determining the mode of interaction between the proteinase and the proteinase inhibitor as they feel that the mechanism is extremely useful.

Activation Date: July 1, 1974

Current Grant Level: $23,793.

George Weinbaum, Ph.D.
Pulmonary Disease Section
Albert Einstein Medical Center
York and Tabor Roads
Philadelphia, Pennsylvania 19141

Lung Proteinase: Antiproteinase Balance and the Effect of Cigarette Smoke on this Interaction.

The twin objectives of this investigation are (1) to identify and quantitate the primary factor responsible for protecting the lung against the action of autogenous proteinases which have been previously shown in the investigator's laboratory to produce experimental emphysema in dogs; and (2) to examine the role of cigarette smoke on this interaction. The researchers shall isolate, purify, characterize, and quantitate the normally occurring substance found in lung tissue which inhibits the activity of specific proteolytic enzymes, and determine if this lung antiproteinase found in dogs has its counterpart in the human lung. The production of antiproteinases and their ability to interact with enzymes capable of inducing experimental emphysema in animals will be studied and evaluated in normal animals and those exposed to cigarette smoke.

The investigator's working hypothesis is that emphysema may be induced by the proteolytic activity of specific enzymes from polymorphonuclear leukocytes and/or pulmonary macrophages. The release of these enzymes may be stimulated by various airborne pollutants. These enzymes overwhelm the local defense mechanisms in the lung, including such factors as serum and tissue antiproteinases, and destroy or alter the elastic tissue of the alveoli.

Activation Date: May 1, 1974

Current Grant Level: $38,163.

Daniel H. Wiseman, M.D.
Los Angeles County -- University of Southern California
Medical Center, Children's Division
1200 North State Street
Los Angeles, California 90033

Persistent Pulmonary Dysfunction Following Specific Lower Respiratory Diseases During Childhood. Grant #647R2

Beginning with the working hypothesis that specific lower respiratory diseases during childhood produce pulmonary injury which can be measured during the acute illness and can be shown to persist even after a complete clinical recovery, this 5-year study is designed for children who experience such acute, severe lower respiratory disease and proposes to show:

A. The acute effects of these diseases upon their pulmonary airway mechanics and ventilation/perfusion relationships.

B. The bacterial and viral pathogens associated with these diseases.

C. The persistence of this pulmonary dysfunction after a complete clinical recovery.

During the first year of this study, over 50 children, four years or younger and without previous history of major respiratory disease, will be selected at random from the hospital admissions. Detailed histories, physical examinations, and chest x-rays will be used to establish clinical diagnosis and estimate the severity of disease. Clinical studies to be employed include viral and bacteriological isolation prior to therapy; arterial blood pH, pCO_2, and pO_2; and urinary nitrogen tension measurements during the acute and convalescent phase of the illness. Clinical and physiological surveillance will be maintained at specific intervals, both before and after discharge from the hospital, until complete physiological recovery criteria are met.

A control group of children admitted for essentially non-respiratory disease will be matched to the study group by age, sex, race or ethnic origin, height, weight, family composition and past history of good health. Their physiological measurements should document the validity of our established normal pulmonary function standards.

Current Grant Level: $22,000.

Other Human Stdys
Lung Dis. & Func

1005075517

Domingo M. Aviado, M.D.
School of Medicine
University of Pennsylvania
36th and Hamilton Walk
Philadelphia, Pennsylvania 19104

Influence of Cigarette Smoke on Pulmonary Emphysema and Bronchospasm.

The object of this project is to examine the significance of cadmium contained in cigarettes which has been implicated as the probably cause of pulmonary emphysema and bronchitis occurring in cigarette smokers.

Mice and rats will be exposed to cigarette smoke with varying concentrations of cadmium. The lungs will be examined functionally and histologically.

At the present time, various strains of mice are being examined to identify those that are susceptible to emphysema or bronchitis.

Activation Date: July 1 - December 31, 1976

Current Grant Level: $19,440.

Jack Chalon, M.D.
Department of Anesthesiology
School of Medicine
New York University Medical Center
550 First Avenue
New York, New York 10016

Epidemiology of Tracheobronchial Multinucleation.

A study is being conducted on the tracheobronchial secretions of patients undergoing general anesthesia for surgery. Smears are made from material suctioned from the endotracheal tube.

A partly prospective and partly retrospective survey on approximately 1,000 patients with a wide variety of prediagnosed extrathoracic malignancies has shown that this population group has an incidence and degree of tracheobronchial epithelial multinucleation significantly higher than a control group matched for age, sex and smoking habit. Cigarette smoking influences the degree of tracheobronchial multinucleation in patients of both sexes with cancer. However, cigarette smoking plays only a minor role in the development of multinucleation, and the single overriding factor affecting this phenomenon is the presence of malignancy. The degree of multinucleation varies with the stage of the growth, being at its highest with early invasive lesions and much lower with intraepithelial cancers and with advanced tumors which invade lymph nodes and distant organs. An epidemiologic survey of approximately 300 patients who were followed up after cancer surgery has shown that a significantly higher recurrence rate was noted in patients who had less than 2.5% multinucleated epithelial cells in smears than in those in which multinucleation reached or surpassed that figure.

Data derived from an extension of the study will be used to devise a test for the diagnosis of occult cancer anywhere in the body and for the prognosis of patients who have undergone cancer surgery.

Activation Date: July 1, 1976

Current Grant Level: $26,772.

Allen B. Cohen, M.D., Ph.D.
Associate Professor of Medicine
Temple University Health Sciences Center
3420 North Broad Street
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The Genetic Defect in Alpha-1-Antitrypsin Deficient Patients.

The purpose of these experiments is to examine the products which result from the interaction of alpha-1-antitrypsin with enzymes in order to determine the mechanism of inhibition. Recent evidence from the investigators' and other laboratories has suggested that enzymes combine with alpha-1-antitrypsin in a manner analogous to the way in which they combine with other protein substrates; however, the alpha-1-antitrypsin enzyme complex becomes frozen in one of the intermediate complexes. Recent evidence in other laboratories has suggested that this complex may be an acyl intermediate. These researchers have been testing this hypothesis. Their evidence to date indicates that alpha-1-antitrypsin combines with trypsin in a 1:1 molar combination. During the process, a peptide is cleaved from alpha-1-antitrypsin. They have accumulated some evidence which suggests that the alpha-1-antitrypsin-trypsin complex is an acyl ester, as suggested by the hypothesis. The complex is alkaline-labeled and separates at pH 9.5 to yield an active enzyme and an inactive fragment of alpha-1-antitrypsin. The new C-terminal residue on alpha-1-antitrypsin appears to be a lysyl residue, suggesting that trypsin combines with a lysyl residue at the inhibitory site of alpha-1-antitrypsin.

The investigators are now performing further experiments to further document this hypothesis. In addition, they are exploring means of affinity labeling the inhibitory sites on alpha-1-antitrypsin so that the active site of the inhibitor can be sequenced.

Activation Date: July 1, 1976

Current Grant Level: $58,730.

Margit Hamosh, Ph.D. (originally Vidic)
Research Associate
Georgetown University
37th & O Streets, N.W.
Washington, D.C. 20007

The Effect of Cigarette Smoke on Lung Metabolism.

Progress Report

1. Lungs from rats exposed to tobacco smoke for five days in the Walton Machine were removed, perfused and ventilated at different rates. Smoked lungs did not increase the incorporation of ^{14}C palmitare with increasing ventilation and became edematous, contrary to controls.

2. Lung slices from rats exposed to tobacco smoke for 30 days showed a 30% decrease in protein synthesis and 100% increase in glycoprotein synthesis.

3. Human lung slices from surgical material ("normal" areas) showed little variation in metabolic activity, regardless of smoking history. However, the metabolic activity of human lung is significantly lower than of the rat.

Current Plans

Further studies on the effect of short-term exposure (less than 5 days) on lung composition and metabolic activity in the rat are in progress.

Activation Date: July 1, 1976

Current Grant Level: $28,175.

Paul Hamosh, M.D.
Assistant Professor of Physiology
& Biophysics and Medicine
Georgetown University Medical School
3900 Reservoir Road, N.W.
Washington, D.C. 20007

The Effect of Smoking on the "Small Airways."

The investigators have measured airway conductance and instantaneous forced expiratory flow rates before and after smoking a Kentucky University research cigarette in healthy young volunteers using: (1) brands with different tar and nicotine content; (2) subjects of different ethnic background; (3) chain smoking (three cigarettes in rapid succession); (4) gases of different density, and (5) filtered vs. nonfiltered smoke. They also performed puff by puff analysis to establish the dose dependence and time course of response.

The researchers have shown so far that airway response to acute smoking is established and maximal after only three puffs; it is independent of the nicotine in the cigarette; it is the same regardless of race or ethnic origin; the range of response is narrow (i.e., no "reactors" were found), and the density-dependence of expiratory flow is unaffected by acute smoking. Filtering the smoke ameliorates, but does not eliminate, the response.

Ongoing research is directed at the effect of acute smoking on the distribution of inspired air.

Activation Date: July 1 - December 31, 1976

Current Grant Level: $14,136.

Clayton G. Loosli, M.D., Ph.D.
Hastings Professor of Medicine & Pathology
School of Medicine
University of Southern California
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Los Angeles, California 90033

Effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of Mice.

This is a continuation of studies of Effects of Fresh Cigarette Smoke Inhalation on the Respiratory Tract of SWR/J, C57L/J and C57Bl/6J male mice. At approximately two months of age, these mouse strains were exposed to fresh cigarette smoke from one cigarette, four times a day, for 17, 21 and 24 months respectively. Compared to lungs of sham and shelf controls, the smoke-exposed mice showed chronic bronchitis, characterized by bronchial epithelial cell hyperplasia, alveolar epithelialization, peribronchial and perivascular round cell infiltration, and alveolar macrophage collections. The lung reactions were most severe in the SWR/J mice and least in the C57Bl/6J mice. Pulmonary adenomas were present in 38% of smoke-exposed and 25% of control SWR mice. However, the control mice were two months younger than the smoke-exposed when sacrificed. Thus, the presence of adenoma in the lungs of smoke-exposed mice could be related more to age and tumor susceptibility than to cigarette smoke. No pulmonary adenomas were present in either smoke-exposed or control C57R/J mice. In 72 C57Bl/6J exposed mice there were six lung tumors (three bronchial adenomas and three alveolar adenomas) and none in 38 controls. More control mice of similar age need to be studied to determine the natural incidence of tumors in this strain.

Studies are planned to see if cigarette smoke inhalation makes animals more susceptible to airborne Sendai virus infections. Also, studies to determine if myxoviruses are cocarcinogenic agents are being planned. Vitamin A deficient and normal C57Bl/6J mice previously given subcutaneous injections of benzpyrene will be, along with controls, subjected to sublethal aerosols of PR8A and Sendai viruses. The post-influenzal nodules will be studied for carcinogenic properties.

Activation Date: April 1 to December 31, 1976

Current Grant Level: $43,394.

Dov Michaeli, Ph.D.
Assistant Professor of Biochemistry & Surgery
School of Medicine
University of California, San Francisco
Third Avenue & Parnassus
San Francisco, California 94143

Effect of Cigarette Smoke on Pulmonary Fibroblasts and Collagen and Its Relation to Emphysema.

The objectives of this project are to:

1. Study the effects of aldehydes on cell division, life span and synthetic activity.
2. Identify the cell fraction to which aldehydes bind.
3. Study the interaction of smoke components with lung matrix molecules and study the immunogenicity of these complexes.
4. Study the thrombogenicity of aldehyde-treated collage.
5. Study the effects of aldehyde-exposed pulmonary macrophages on platelets.

All the stated experiments will be conducted with C^{14}-labeled formaldehyde. This will enable the investigators to quantitatively measure the phenomena they intend to observe. Lung fibroblasts (WI 38) and pulmonary macrophages in tissue culture will be exposed to aldehydes. Their effects on the macrophages themselves and on platelets will be measured by monitoring the release of proteolytic enzymes as well as the release of vasoactive amines by the platelets.

Activation Date: July 1, 1976

Current Grant Level: $74,000.

Lynne M. Reid, M.D.
Chairman, Department of Pathology
Children's Hospital Medical Center
300 Longwood Avenue
Boston, Massachusetts 02115

The Effects of Irritation and Drugs on Airway Epithelium -- An Experimental Study of Mechanisms.

The airway epithelium, part of the surface of the body, is affected directly by irritants and pollutants. Two aspects of this response are of major importance to human disease -- (1) hypersecretion of mucus accompanied by increase in the number of mucus-secreting cells and (2) disturbance of cell division. These responses can be linked respectively to chronic bronchitis and bronchial carcinoma, two conditions which have been linked to the habit of tobacco smoking.

From an understanding of the normal epithelium, its structure and metabolism, the response to tobacco smoke can be analyzed into those features that are specific to it and those that are common to "irritants" in general.

The use of anti-inflammatory agents has afforded protection against certain of the effects of tobacco smoke.

In this study, rats will inhale freshly-produced tobacco smoke and its effect will be compared with drugs stimulating secretion. The way anti-inflammatory agents and neuromimetic drugs modify the changes produced by tobacco will indicate the mechanisms producing the response to irritation.

Activation Date: July 1, 1976

Current Grant Level: $100,000.

Nathan H. Sloane, Ph.D.
Department of Biochemistry
University of Tennessee
894 Union Avenue
Memphis, Tennessee 38163

Effect of Benzo(a)pyrene and Derivatives on Mammalian Lung Cells.

These investigators have determined that the rat enzyme, 6-hydroxymethylbenzo(a)pyrene is present in the lung and liver both as a membrane-bound form and as a soluble form. The soluble lung enzyme has been purified about 200-fold; the C-1 compound is bound to the enzyme and is not removed by Dowex I or released by Aminopterin treatment. The synthetase reaction proceeds via a pathway not involving cytochrome P-450.

The researchers will utilize tissue culture techniques to study the role of nonoxidative pathways in cell transformation utilizing mammalian lung cells.

Activation Date: July 1, 1976

Current Grant Level: $14,562.

James Travis, Ph.D.
Associate Professor of Biochemistry
University of Georgia
Athens, Georgia 30602

Biochemistry of Chronic Obstructive Lung Disease.

The objective of this proposal is to determine the roles in tissue proteolysis of -1 proteinase inhibitor (-1-antitrypsin), granulocytic elastase and other proteases from granulocytes and macrophages.

In particular, the investigators wish to determine what structural differences account for the inability of ∂-1-PI type ZZ (and also type MZ) individuals to secrete sufficient amounts of inhibitor to combat proteolysis. They would also like to know the mechanism by which the inhibitor normally functions in order to possibly develop synthetic inhibitors. Finally, they would like to determine how the granulocytic and macrophage proteases function, in order to, again possibly, develop better synthetic inhibitors to these enzymes.

In this respect the researchers hope to extend studies of this system to an investigation of the development of emphysema in individuals with normal ∂-1 PI levels. Thus, in individuals who inhale large quantities of particulate matter (i.e., cigarette smoke), are macrophage proteases being released in quantities large enough to overwhelm the normal defense mechanism or does the macrophage actually produce proteases which can digest lung tissue but which are not inhibitable by ∂-1-PI?

Activation Date: July 1, 1976

Current Grant Level: $45,419.

Gerald M. Turino, M.D.
Professor of Medicine
College of Physicians & Surgeons
Columbia University
630 West 168th Street
New York, New York 10032

Chemical Basis of Tissue Destruction in Obstructive Lung Disease.

This study is designed to define the role of elastases of alveolar macrophages and of circulating polymorphonuclear leukocytes in the development of pulmonary emphysema in human subjects. Assays of elastase activity will be performed using an oxalate-treated elastin substrate developed in the investigator's laboratory. Leukocytes are obtained by phlebotomy and macrophages will be harvested by bronchoscopic lavage from several patient groups: (1) normal subjects with isolated radiologic lesions requiring bronchoscopy; (2) patients with chronic obstructive lung disease whose physiological and clinical manifestations are primarily those of pulmonary emphysema, and (3) patients with chronic obstructive lung disease with characteristics primarily of bronchitis. Distinctions will also be sought between smokers and nonsmokers. Elastase activity in macrophages from animals with experimental emphysema induced by proteolysis will be assayed also.

The effects of reagents such as cytochalasian, colchicine and thioglycollate on the levels of macrophage elastase activity will be studied in tissue culture of alveolar macrophages. These agents produce marked changes in elastase activity in peritoneal macrophages. Chloromethylketone derivatives which inhibit pancreatic and leukocyte elastase will be studied for inhibition of alveolar macrophage elastase.

The ultimate purpose of these investigations is to evaluate the potential role of alveolar macrophage elastase activity as an etiological agent in tissue destructive changes in airway obstructive diseases of the lung.

Activation Date: July 1, 1976

Current Grant Level: $32,359.

George Weinbaum, Ph.D.
Pulmonary Disease Section
Albert Einstein Medical Center
York and Tabor Roads
Philadelphia, Pennsylvania 19141

Lung Proteinase: Antiproteinase Balance and the Effect of Cigarette Smoke on this Interaction.

The twin objectives of this investigation are (1) to identify and quantitate the primary factor responsible for protecting the lung against the action of autogenous proteinases which have been previously shown in the investigator's laboratory to produce experimental emphysema in dogs; and (2) to examine the role of cigarette smoke on this interaction. The researchers shall isolate, purify, characterize, and quantitate the normally occurring substance found in lung tissue which inhibits the activity of specific proteolytic enzymes, and determine if this lung antiproteinase found in dogs has its counterpart in the human lung. The production of antiproteinases and their ability to interact with enzymes capable of inducing experimental emphysema in animals will be studied and evaluated in normal animals and those exposed to cigarette smoke.

The investigator's working hypothesis is that emphysema may be induced by the proteolytic activity of specific enzymes from polymorphonuclear leukocytes and/or pulmonary macrophages. The release of these enzymes may be stimulated by various airborne pollutants. These enzymes overwhelm the local defense mechanisms in the lung, including such factors as serum and tissue antiproteinases, and destroy or alter the elastic tissue of the alveoli.

This work has been reported in:

Weinbaum, G., Takamoto, M., Sloan, B., Meranze, D.R. and Kimbel, P. Lung antiproteinase: a possible primary defense against emphysema development. American Review of Respiratory Disease 109:741, 1974.

Weinbaum, G., Takamoto, M., Sloan, B. and Kimbel, P. Partial purifications and characterization of a lung antiproteinase and its differentiation from serum antiproteinases. Abstracts, Aspen Lung Conference. Chest 67(Suppl. 2):31-32, 1975.

Weinbaum, G., Takamoto, M. and Kimbel, P. Further characterization of canine lung antiproteinase and isolation of a similar inhibitor from peripheral human lung. American Review of Respiratory Disease (in press, 1975).

Activation Date: May 1, 1976

Current Grant Level: $42,838.

Jack Chalon, M.D.
Department of Anesthesiology
New York University Medical Center
School of Medicine
550 First Avenue
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Changes in Tracheobronchial Cytology.

The objectives of this investigation are:

(1) The correlation of preoperative lung functions, intraoperative tracheobronchial cytology and postoperative pulmonary complication rate, to see how effective cytology is in predicting lung damage in smokers.

(2) The correlation of tracheobronchial cytologic changes related to female sex hormone blood levels with the morphologic integrity of ciliated cells to assess the degree of protection afforded by hormones against damage caused by smoking.

(3) The discovery of cytologic changes associated with malignancy for the diagnosis of bronchogenic carcinoma, and in order to learn the effects of extrapulmonary malignancies on tracheobronchial epithelium.

The following procedure will be used:

(1) Collection of tracheobronchial smears from patients undergoing general anesthesia for surgery.

(2) Scoring of damage to the ciliated cells by a point system in nonsmokers and various smoking categories.

(3) Scoring of changes in the ciliated cells induced by variations in blood levels of circulating sex hormones and the correlation of results obtained from the above.

(4) Assessment of preoperative lung functions (PEFR, FEV_1 and FEV_3) in nonsmokers and smokers and correlation with results obtained in (2) above with postoperative complication rate and with lung function tests.

Current plans are to continue all previous studies and to try and explain the results obtained by (1) cell cultures, (2) chemical changes discovered in the serum of patients concerned, and (3) biochemical analysis of the changes noted.

1005075530

Activation Date: July 1, 1974

Current Grant Level: $18,492.

Sanford Chodosh, M.D.
Tufts University School of Medicine
136 Harrison Avenue
Boston, Massachusetts

Chronic Bronchitis Entities.

The careful and thorough evaluation of a representative number of patients who are currently categorized as having chronic bronchitis (CB) is being continued at the Tufts Lung Station at the Boston City Hospital. The purpose is to determine the criteria for delineating the possible subgroups or new disease entities which may now be included in the CB group, and to clarify the pathophysiology of each of these. Out-patients with chronic productive cough are evaluated using multidisciplinary techniques. Clinical studies include careful histories and physical examinations. Physiologic studies include spirometry, intrapulmonary mixing, gas exchange, blood gases, and plethysmography. Radiology includes laminograms and bronchograms. Sputum evaluations include volumes, cytology, physical characteristics, bacteriology, and chemistry. Routine laboratory tests, electrocardiograms, and allergy testing are also included. Additional histochemical and biochemical studies of sputum and lung tissue are planned in cooperation with the Science Resources Foundation. The data is being collected on statistical forms prepared for this study. Routine, multivariant and discriminant analyses are underway in cooperation with Kurt Enslein of Rochester, New York. Incidental information is anticipated in that diagnostic and prognostic criteria applicable to chronic obstructive lung diseases should be elucidated.

Activation Date: January 1, 1971

Current Grant Level: $109,194.

Allen B. Cohen, M.D., Ph.D.
Department of Medicine
School of Medicine
University of California, San Francisco
3rd and Parnassus
San Francisco, California 94143

The Genetic Defect in Alpha-1-Antitrypsin Deficient Patients.

The aim of this project is to establish the genetically determined biochemical differences between alpha-1-antitrypsin from normal subjects and that of patients deficient in this protein.

Alpha-1-antitrypsin will be isolated from both groups of subjects. Peptide maps and amino acid analysis will show if there is a genetically determined difference in the amino acid moiety of the proteins.

The project starts within one week. Purification of the normal protein has been achieved. Purification of the abnormal protein will be undertaken.

Activation Date: May 1, 1974

Current Grant Level: $41,000.

John R. Esterly, M.D.
Department of Pathology
University of Chicago
950 East 59th Street
Chicago, Illinois 60637

Resolution of Pulmonary Injury.

The project is designed to investigate the sequellae of injury to the peripheral lung (respiratory bronchioles, alveolar ducts, and alveoli) and determine the type and severity of histochemical and morphologic changes which are correlated with complete or partial structural resolution. The morphologic and histochemical characteristics of replicating alveolar epithelial cells will be determined, as well as the variation in the alveolar exudate following injury with chemical and biologic agents.

Activation Date: October 1, 1972

Current Grant Level: $30,028.

Aaron J. Ladman, Ph.D.
The University of New Mexico School of Medicine
915 Stanford Drive N.E.
Albuquerque, New Mexico 87106

Effect of Cigarette Smoking on Lipids and Morphology of Alveolar Lining Material and Macrophages. Grant #628R2

Four studies are being undertaken:

(a) Determination of the effect of cigarette smoking on the lipids and morphology of alveolar lining material and macrophages recovered by saline lavage of smoking and non-smoking humans.

(b) Determination of acute and chronic effects of smoking on lipids and morphology of dog alveolar lining material and macrophages in situ as well as on fractions recovered by saline lavage.

(c) Determination of the effect of cigarette smoking on the incorporation of radioactive lipids into alveolar lining material and macrophages of the dog.

(d) Determination of the origin of the alveolar macrophage by labeling circulating white cells with radioactive lipids and amino acids and monitoring the transport of these labeled cells into the alveoli of the lung by radioautographic microscopy.

Current Grant Level: $26,795.

Illinois Institute of Technology Research Institute
10 West 35th Street
Chicago, Illinois 60616

Effects of Tobacco Smoke Inhalation on Lung Surfactant and Alveolar Macrophages.

The research into the inhibition effects of gaseous components of cigarette smoke on the phagocytic activity is being continued. Smoke effects are being evaluated by the measurement of phagocytosis, cell adhesion to glass, cytochrome redox and other appropriate indicators of the aerobic metabolic state. The effects of catecholamines upon the phagocytic capacity of macrophages demonstrated by spectral shifts of the cytochromes, inhibition of mobility, etc., are also under investigation. Initial studies of relationship of smoke to impairment of physiological function are underway and radio tracer tagged particles of 0.5μ to 1.2μ size are being developed to expedite the assay of particle incorporation (phagocytosis).

Activation Date: July 1, 1972

Current Contract Level: $73,272.

Joseph M. Lauweryns, M.D., Ph.D.
Experimental Laboratory of Cardiopulmonary
and Genital Pathology
Vesalius Institute
University of Leuven
12, Minderbroedersstraat, 3000
Leuven
Belgium

The Lymphatics of the Lung: Their Role in Fluid Transport and Clearance of Airborne Particulate Matter.

The morphology of the pulmonary lymphatics and their role in pulmonary fluid transport and airborne particulate matter will be investigated, using light optical and electron microscopical methods.

Activation Date: January 1, 1971

Current Grant Level: $37,539.

Clayton G. Loosli, Ph.D., M.D.
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Los Angeles, California 90033

The Effects of Inhalation of Whole Smoke, Synthetic Smog, Ambient Air, Alone and in Combination, on the Respiratory Tract and Other Organs of Pathogen-Free Male and Female Mice.

This study will concern itself primarily with non-neoplastic lung tissue changes; however, careful observations will be made for evidence of pre-neoplastic and neoplastic changes in the lungs and other organs of the smoking and non-smoking mice. The specific aim of the study is to compare lung reactions and changes in other organs (if any) in pathogen-free male and female mice exposed to "synthetic smog" and cigarette smoke, alone and in combination. The following environmental exposures will be employed:

(a) Charcoal filtered air alone (controls).
(b) Charcoal filtered air plus "synthetic smog" (non-smoking animals).
(c) Charcoal filtered air plus "synthetic smog" (smoking animals).
(d) Charcoal filtered air alone for smoking animals.

Based on these exposures, observations will be made for the following purposes:

1. To determine any histological changes which may occur in the respiratory tract (buccal cavity, larynx, trachea, bronchi and respiratory portion), as well as in the spleen, liver, kidney, adrenals, and possibly brain of male and female mice (COBS) exposed to whole smoke or "synthetic smog" under controlled laboratory conditions.

2. To determine if histological changes which may occur in the respiratory system and other organs of COBS mice are additive (more severe) when subjected to environments of "synthetic smog" and whole smoke in combination.

3. To compare the histological changes in the respiratory tract of male and female mice when subjected to environmental exposures. While the lungs of the COBS strain of mice are "beautifully clean," it is expected that changes will occur as a result of exposure to "synthetic smog," cigarette smoke, or to a combination of both, compared to the lungs of mice living in filtered air. Several procedures will be employed to evaluate these changes and include (a) tritiated thymidine for cell turnover, (b) comparative counts of type II alveolar cells in IDH stained sections, (c) determination of collagen (hydroxyproline) content of lungs, and (d) use of fluorescent procedures to localize fluorescent materials from cigarette smoke.

Activation Date: October 1, 1971

1005075537

Current Contract Level: $98,223.

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Elastolytic Breakdown in the Etiology of Pulmonary Emphysema. Grant #546-AR1

The main emphasis of this study will be placed on establishing the etiology of pulmonary emphysema by experiments designed to adduce evidence for our working hypothesis that excessive availability of elastolytic enzymes causes damage to lung elastin which leads to loss of elasticity. In familial panlobular emphysema a deficiency gene has been found responsible for low titers of serum inhibitors for trypsin, elastase and other proteolytic enzymes. Patients with this condition are being screened as well as their relatives and the identify of the factors involved will be ascertained. At the same time the specific enzyme, probably leucocyte in origin, which is inhibited in normal individuals and free to act in the emphysema patients is sought. An animal model has been established in rats which are made emphysematous by injection of elastolytic enzymes. Induction of the disease can be prevented by progesterone injections. The mechanism leading to the establishment or the prevention of the disease will be explored further. Lung tissue obtained from autopsy specimen and from experimental animals will be studied with special emphasis on the changes in composition of elastin from normal and emphysematous persons and/or animals.

Current Grant Level: $33,875.

Dov Michaeli, Ph.D.
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Effect of Cigarette Smoke on Pulmonary Fibroblasts and Collagen and Its Relation to Emphysema.

The objects of this project are to:

a. Study the immunogenicity of cigarette smoke components complexed with lung collagen by the radioimmunoassay (RIA) technique.

b. Study the effects of components of the gaseous phase of cigarette smoke on the division rate of lung fibroblasts, and on the synthesis and secretion of collagen and mucopolysaccharides.

c. Identify the cellular site(s) of action of cigarette smoke components.

d. Study the chemical interactions between cigarette smoke components and matrix macromolecules, and the possible effect of such interactions on the biosynthetic activity of lung fibroblasts.

e. Study the immunopathology of emphysematous lungs using immunoelectron-microscopic techniques.

To investigate these objectives, a multidisciplinary approach will be taken. Lung collagen will be reacted with aldehydes present in the gaseous phase of cigarette smoke and antibodies against this antigen will be produced in experimental animals. The antigen will also be assayed for in sera of patients with emphysema. In another approach, lung fibroblasts (WI 38) will be exposed to aldehydes of the gaseous phase of cigarette smoke, and its effect on their synthetic and proliferative activity will be tested. Synthetic activity will be measured by assay of synthesis of new collagen. Proliferative activity will be assayed by uptake of radioactive thymadine. The cellular sites of action of cigarette smoke components will be studied using radioactive aldehydes and cell fractionation.

The investigators are in the process of obtaining antibodies to the aldehyde-triggered collagen. Experiments on the localization of aldehydes in cell components are underway. A radioimmunoassay for lung collagen has been perfected in their laboratory and is currently in use.

Activation Date: July 1, 1974

Current Grant Level: $67,000.

Albert H. Niden, M.D.
Department of Medicine
Director, Pulmonary Disease Section
Drew Postgraduate Medical School
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Effects of Cigarette Smoke, Noxious Fumes and Drugs on the Terminal Airways.

The aims of this project are:

(1) To elucidate the role of the large alveolar cell, nonciliated bronchiolar cell, and monocyte-macrophage system in pulmonary phospholipid metabolism. Phospholipid metabolism will be studied by utilizing autoradiographic techniques to localize and follow the subcellular formation of phospholipids from fatty acid precursors. The effect of various stimuli, e.g., cigarette smoke, noxious fumes, and drugs, on phospholipid metabolism by these cells will be observed.

(2) To study pulmonary capillary permeability and the factors affecting it.

By using E.M. tracer protein molecules of known sizes (horseradish peroxidase at 40 Å, hemoglobin at 65 Å, Dextran at 40 Å to 200 Å), one is able to investigate the effects of intravascular pressure and damage to the capillary endothelium on pulmonary capillary permeability. The effects of air pollutants, drugs, and cigarette smoke on pulmonary capillary permeability will thus be studied.

Activation Date: January 1, 1974

Current Grant Level: $25,618.

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Effect of Benzo(a)pyrene and Derivatives on Mammalian Lung Cells.

The objectives of this project are:

(1) To determine whether metabolic hydroxymethylation of benzo(a)pyrene to the 6-hydroxymethyl derivative represents a pathway for the formation of a more proximate carcinogen; and

(2) To determine levels of benzo(a)pyrene metabolizing enzymes in the lungs of mice susceptible and resistant to chemical carcinogens.

The first step will be the determination of mouse lung levels of both the aryl hydroxymethyl synthetase and the aryl side chain methyl hydroxylase, that converts 6-methyl benzo(a)pyrene to the hydroxymethyl derivative.

Activation Date: July 1, 1974

Current Grant Level: $12,893.

terminated

David M. Spain, M.D.
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Pulmonary Parenchymal Alterations in an Autopsied "Normal" Population as Related to Age, Sex, and Environmental Factors. Grant #678R1

Whole lung sections will be obtained from otherwise healthy individuals dying from accidents, suicides, and homicides immediately or shortly thereafter so that secondary or underlying lung alterations of a chronic nature will be kept to a minimum. A quantitative study of emphysema and vascular changes will be made on these preparations and findings will be related to age, sex, occupation, place of residence, and smoking habits. Findings will also be related to an on-going study (supported by NIH) in which material, obtained in a similar manner, is being processed so that tracheal bronchial alterations can be studied.

It is hoped that the information obtained can provide a baseline frame of reference for those studies that have been performed on autopsied hospital populations which are necessarily biased.

Current Grant Level: $21,390.

John V. Weil, M.D.
University of Colorado Medical Center
Denver, Colorado

Effects of Cigarette Smoking and of Chronic Airway Obstruction on Hypoxic Ventilatory Drive in Man. Grant #706R1

A new method has been devised for measurement of hypoxic ventilatory drive in man. A continuous curve relating alveolar oxygen tension and minute ventilation is inscribed with alveolar carbon dioxide tension held constant. Using this method effects of acute and chronic cigarette smoking will be studied in man. Independent effects of chronic airway obstruction will also be assessed. Nicotine is a well-known stimulant of peripheral chemoreceptors and these studies should indicate whether this fact has implications for chemoreceptor function in smoking man.

Current Grant Level: $29,724.

Branislav Vidic, M.D.
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The Effect of Cigarette Smoke on Lung Metabolism.

The principal objectives of this research are to study the effect of cigarette smoke on (1) the biosynthesis of surfactant and connective tissue in the lung; and (2) the mechanical properties of the lung.

Rats will be chronically exposed to cigarette smoke and then subjected to three main experimental designs. The first aims to study the incorporation and turnover of labeled substrates presented to the intact animal. The second calls for manipulation of an isolated ventilated and perfused preparation of the lung, stressed mechanically and/or biochemically. The investigators will study the effect of these acute stresses on the biosynthesis and turnover of substrates in the lung. The third design involves isolation of the great alveolar cell and the study of in vitro surfactant synthesis.

The methodology has been worked out for measuring all desired parameters of lipid and protein metabolism. Preliminary studies were made on the effect of forced ventilation, and acute and chronic administration of substances with a known effect on the metabolism of the lung (e.g., cortisone). Actual exposure to smoke was started recently.

Activation Date: January 1, 1975

Current Grant Level: $40,000.

EPIDEMIOLOGY

Raymond Bosse, Ph.D. (formerly Charles L. Rose, M.D.)
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Normative Aging Study
Veterans Administration Outpatient Clinic
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A Smoking Research Project in the Normative Aging Study.

The objective of this research program is to study the relationships between smoking behavior and the aging process.

Specifically, the smoking research program is relating in-depth smoking data to extensive biomedical and behavioral parameters in a population of 2,000 male veterans. The study is also longitudinal, such that changes over time are also being analyzed. Smoking data is collected both by self-administered mail questionnaires and by medical interviews.

The following findings have been reported. Smoking was found to relate to decreasing pulmonary function values with increasing age. Although giving up cigarettes accounted for subsequent weight gain, age was found to be principally responsible for the weight gained. The personality dimensions of anxiety and extroversion were found to be related to smoking. Empirical support was also found for a typology of smoking based on management of affect. Six types of smoker motives were thus suggested.

During the current year, investigators plan to relate smoking status to longitudinal changes in biomedical data. Also, a greater refinement of smoker motives will be attempted and an attempt will be made to better relate motives for smoking to both behavioral and biomedical outcomes.

Activation Date: July 1, 1976

Current Grant Level: $64,000.

Paul T. Costa Jr., Ph.D.
Associate Professor of Psychology
University of Massachusetts at Boston
Harbor Campus
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The Relations between Smoking Motives, Personality and Feelings.

This research will examine smoking behavior and motivations in the context of personality structure, life change and stress. In particular, an attempt will be made to distinguish psychologically adaptive from maladaptive smoking.

A series of self-report instruments measuring personality, life stress, smoking motivation and mood will be administered to 1,050 adult males, participants in an ongoing longitudinal study of nonpathological aging. These data will be analyzed in conjunction with existing psychological, sociodemographic and biomedical data. Longitudinal analyses will be used, along with multivariate statistical techniques of cluster and factor analysis.

Previous work has resulted in the identification of three major dimensions of personality: anxiety-adjustment, extraversion-intraversion, and openness to experience. Research efforts on measuring smoking motives have been based on classifying the functions which smoking serves for the individual derived from the reasons given for smoking. Six factors or motives have been consistently identified which show differential relations to the three personality dimensions and actual smoking behavior.

During the current year, research will focus on data collection and the refinement of instruments. Specifically, participants will be surveyed as to the time, place and circumstances in which they smoke. Additionally, the longitudinal stability of motives for smoking will be examined and a motive-based typology of smokers will be developed. Different smoker motive-based typology of smokers will be developed. Different smoker types will be characterized in terms of mood and personality dimensions, as well as age and sociodemographic status.

Activation Date: July 1, 1976

Current Grant Level: $42,228.

David W. Crumpacker, Ph.D.
Professor and Chairman
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and Organismic Biology
University of Colorado
Boulder, Colorado 80309

Genetic and Environmental Factors Affecting Smoking Behavior.

The main aim is to conduct a comprehensive and detailed analysis of the degree to which genetic and environmental factors are involved in the determination of smoking behavior. A pilot study of randomly-sampled adults will be conducted in Denver, Colorado. This will be followed by a much larger study of adult Swedish twins and their adult relatives. In both cases, information will be obtained from a set of individually administered questionnaires dealing with smoking behavior and other constitutional and environmental variables.

Subsequent analyses will be based on a path model that relates genetic and environmental effects. Primary emphasis will be placed on obtaining unbiased estimates of narrow-sense heritability for various components of smoking behavior. Estimates of parameters associated with nonadditive genetic variance, assortative mating, prenatal environment and postnatal environment will also be obtained. A broad-sense heritability estimate will be constructed which pertains to the Swedish urban population in the range of environments investigated.

In addition, studies will be conducted on (1) the interrelationships of smoking, constitutional and socioeconomic variables; (2) changes of smoking behavior with time, and (3) methodologies to be used for analyses of smoking behavior traits more complex than those of smoking incidence and tobacco consumption.

Activation Date: June 1, 1976

Current Grant Level: $197,128.

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Characteristics of Smokers and Nonsmokers.

The goal of these investigations is to determine the extent to which differences exist between smokers, nonsmokers and exsmokers with respect to a large number of variables (socioeconomic, morphological, biochemical, physiological, psychological and social history) in a large heterogeneous population studied by automated multiphasic screening during the period 1964-1973.

Persons have been classified according to smoking habits as determined from a self-administered questionnaire. The distributions of the other characteristics, studied singly and in combination, are being compared in the different smoking habit groups.

Several studies on the relationship of smoking to a variety of characteristics have been completed and published. Current emphasis is on characteristics related to persisting in vs. cessation of cigarette smoking, taking advantage of longitudinal data on persons receiving multiple examinations. This year the investigators will also begin a study on the relation of smoking habits to mortality.

Activation Date: May 1, 1976

Current Grant Level: $99,820.

Benjamin Bell, M.D.
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A Smoking Research Program in the Normative Aging Study.

The objectives of this program are (a) to improve smoking research data in the Normative Aging Study through the collection of more extensive and in-depth smoking data; and (b) to study extensive ramifications and antecedents and effects of smoking, both behavioral and biomedical.

The investigators lean heavily on mail questionnaires for detailed smoking data and related behavioral data. They also stress interdisciplinary analysis spanning behavioral and biomedical measures, both cross-sectional and longitudinal. In addition, they construct extensive computerized data files which facilitate analyses of large-scale data using complex multivariate methods.

During the next year, the researchers plan studies relating smoking to drinking, lifestyles, neurological assessments and other biomedical parameters.

Activation Date: July 1, 1974

Current Grant Level: $64,000.

H. J. Eysenck, Ph.D., D.Sc.
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England

The Inheritance of the Smoking Habit.

The objective of the proposed study is to investigate the heritability of the tobacco smoking habit. Previous studies have not made use of modern methods of biometric genetic analysis, and hence do not enable us to answer the question. The proposed study seeks not only to partition the variation in the population into environmental and genetic causes, but also to provide information on the type of gene action involved, and to permit a statistical test of the adequacy of the assumptions underlying the genetic model to be used. An additional objective of the study is to discover the degree to which the genetic and the environmental parts of the individual's smoking behavior are determined by personality factors, and to what extent these are inherited.

The investigators' approach is through the analysis of questionnaire answers furnished by members of their large twin register, and by the families of the twins. Also being investigated are groups of foster children, now grown up, and their foster parents, as well as large groups of related subjects, which are enabling the researchers to carry out familial analyses.

The collection of responses from the twins is now almost complete, and that of the foster children and parents is about half complete; in the coming year most attention will be given to the familial study.

Activation Date: April 1, 1974

Current Grant Level: $21,000.

Ilari Rantasalo, M.D.
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The Finnish Twin Registry.

The main goal of this study is to elucidate the role of environmental factors in the manifestation of diseases and in mortality, using a twin registry which enables standardization of the genetic factors. The groups of diseases of primary interest are: (1) degenerative coronary diseases; (2) degenerative disorders of cerebral circulation; (3) chronic pulmonary diseases: bronchitis and emphysema; and (4) malignant tumors: cancer of the lung, ventricle, rectum, breast and cervix. The environmental factors of primary interest are: (1) smoking; (2) use of alcohol; (3) psychic and social stress factors; (4) anamnesis of training, family and work; and (5) physical activity.

Size of register: All twin pairs of the same sex living in Finland, born 1957 or before. Sources of data: The computerized personal register of the State, which is the source of the addresses of the twins. The main part of the basic information will be obtained from mailed questionnaires. The main means of the follow-up of morbidity and mortality are the computerized registeries: the hospital register and the register of the causes of death.

The program of the first year is to establish the address register and to mail the first questionnaire with coding and punching the answers received and to start the follcw-up.

Activation Date: January 1, 1975

Current Grant Level: $75,000.

NICOTINE PHARM.

1005075553

J. P. Long, Ph.D.
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Sympathomimetic Action of Nicotine. Grant #419-AR2

Various experiments have been conducted in attempting to elucidate the mechanism of action of nicotine in activating the sympathetic nervous system. Results of these works include suggestions such as (1) central, (2) ganglionic stimulation, (3) ramifying ganglia, and (4) direct receptor activation. We are completing a project using the guinea pig vas deferens and we are able to demonstrate that (1) EM examination demonstrates that there are no ganglia within the tissue and (2) nicotine produces contracture that is blocked by C-6, bretylium, guanethidine, etc. We are now labeling the nerve terminals with tritium labeled norepinephrine followed by the application of nicotine. It appears that nicotine is producing contracture of the vas deferens by releasing norepinephrine from the nerve terminals. I believe that we are accumulating evidence that nicotine may be acting on this tissue by a mechanism other than a classical nicotinic action. We are continuing our work with DMAE in attempting to elucidate the potent antinicotinic action of this compound. We have demonstrated that it is not a "ganglionic blocker." It does block the norepinephrine releasing properties of nicotine using the vas deferens. Using nerve tissue it produces an efflux of Ca^{45}, but as yet we have no idea of which Ca^{+} pool. We will attempt to unblock the nicotine blocking action of DMAE by the administration of calcium.

Current Grant Level: $14,432.

Hector C. Sabelli, M.D.
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Electrophysiological and Biochemical Correlates of Nicotine Action with Special Reference to the Central Nervous System. Grant #472R4

This continuing project is part of a long-term and broad program in neuropharmacology, aimed at the analysis of the central synaptic actions of drugs and their correlation with effects upon behavioral parameters. The central effects of nicotine are studied in comparison to and in interaction with relevant cholinergic, serotonergic and adrenergic agents in an effort to study separately its cholinomimetic and catecholamine releasing actions. A battery of behavioral and electrophysiological techniques are used but in the present series of experiments special emphasis is given to intracellular recordings in cortex and nerve. Biochemical techniques will be used to differentiate the role of various monoamines and their aldehyde metabolites on nicotine action.

Current Grant Level: $20,020.

Thomas C. Westfall, Ph.D.
University of Virginia School of Medicine
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Influence of Nicotine and Related Drugs on the Uptake, Storage, Release and Turnover of Catecholamines in Central and Peripheral Tissue. Grant #467-A

The present experiments are an extension of previous work which indicates that nicotine and related drugs produce some of their cardiovascular and central effects through adrenergic mechanisms. The present studies will take into consideration the dynamic aspects of norepinephrine (NE) storage in peripheral and adrenergic neurones. The influence of drugs on NE turnover will be carried out by 3 methods:

(1) By following the decline in specific activity of labeled NE from the heart following the IV administration of NE.

(2) By measuring the disappearance of endogenous NE following inhibition of synthesis by tyrosine hydroxylase inhibitors.

(3) By measuring the conversion of labeled precursors, such as tyrosine and dopa, to NE.

These studies will be performed on the perfused guinea pig and rat heart. Labeled NE will be perfused in the absence or presence of nicotine and related drugs. Studies on the effect of nicotine on the uptake and turnover of NE in central tissue will be carried out following the direct application of labeled NE into the lateral ventricles of rats.

Current Grant Level: $27,913.

MISCELLANEOUS

1005075557

Alphonse J. Ingenito, Ph.D.
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Actions of Carbon Monoxide and Tobacco Smoke on Retinal Metabolism and Function.

The objective is to study the effects of carbon monoxide (CO), nicotine and tobacco smoke on retinal function and metabolism as determined by effects of CO on the dark-adapted electroretinogram (ERG) of the chloralose-anesthetized cat, on an isolated rabbit retina-optic nerve preparation, and on various incubated in vitro preparations of rabbit retina or bovine pigment epithelial cell suspensions. These latter are designed to determine primarily whether CO can affect the ability of the retinal pigment epithelium to convert retinol to retialdehyde. The effects of CO on other general metabolic functions, e.g., glucose uptake and lactate production are also being studied in the incubated rabbit retina.

Present data indicates that CO causes a significant decrement in b-wave amplitude of the ERG at blood carboxyhemoglobin levels in the "smoking range," i.e., less than 10%. Studies with tobacco smoke, nicotine and nicotine-CO combinations on the ERG of the anesthetized cat reveal that nicotine has relatively little effect on the ERG and that the CO complement in tobacco smoke plays a significant role in the effects of tobacco smoke on the ERG of the anesthetized cat. There does not appear to be a significant interaction between the effects of nicotine and CO. The data could have some significance in elucidating the etiology of "tobacco amblyopia."

Activation Date: July 1, 1976

Current Grant Level: $24,815.

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A Study of the Effects of Nicotine on Gestation in the Rat with Particular Reference to Implantation and the Time of Onset of Parturition.

Considerable evidence indicates that nicotine adversely affects pregnancy in the rat. Among such disruptive effects are increased frequency of fetal resorption, reduced litter size and weight, and delayed parturition. The primary objective of the experiments embodied in this project is to gain a greater understanding of the means by which nicotine exerts disruptive effects on the normal course of pregnancy, and more particularly, how the chief alkaloid of tobacco reduces litter size and delays parturition. To this end, a variety of parameters will be measured to assess reproductive efficiency, time of implantation, fetal growth and development, and normalcy of parturition in nicotine treated vs. control rats.

Several experimentally-testable hypotheses are proposed. Specifically, experiments will be conducted to determine if nicotine-induced reduction in litter size and delay in parturition are related to alterations in uterine sensitivity to implantation. The time of onset, magnitude, and duration of sensitivity to decidualization will be compared in treated and control rats. In addition, the effects of nicotine on duration of gestation will be determined by an accurate measurement of the time of parturition.

Activation Date: June 1, 1976

Current Grant Level: $6,582.

B. V. Rama Sastry, D.Sc., Ph.D.
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Influence of Nicotine on the Release of Acetylcholine in the Human Placenta and its Implications on the Fetal Growth.

As a part of this project, the existence of a unique cholinergic system in human placenta was demonstrated. This system contains acetylcholine (ACh), choline acetyltransferase (ChA, the enzyme that catalyzes the synthesis of ACh from acetylcoenzyme A and choline) and acetylcholinesterase (AChE, the enzyme that hydrolyzes ACh into acetate and choline). This ACh-ChA-AChE system is unique because there is no evidence for the presence of nerves in the human placenta. Moreover, it is localized in the syncytiotrophoblast, a cell layer which separates the maternal circulation from fetal circulation.

Nicotine showed a biphasic effect on the rate of ACh release from the isolated placental villus. Thus: at 5.8×10^{-6} M of nicotine there was no effect; at 5.8×10^{-5} M of nicotine the rate of ACh release increased by about 40%; and at 7.7×10^{-4} M the rate was depressed by about 30%. The stimulatory effect of nicotine was depressed significantly by atropine, but not by d-tubocurarine. It was suggested that the release of ACh by nicotine was probably mediated through a cholinergic receptor of the muscarinic type.

The future studies will be aimed at (a) the mechanisms of ACh release by nicotine from placenta, (b) the effects of carbon monoxide and anoxia on the ACh release, uptake of nutrients (amino acids and sugars) by the placental villus, and (c) the effects of anoxia on the lactic acid production which in turn may participate in the formation of lactoyl-CoA and lactoylcholine ("a false local hormone?")

Activation Date: May 1, 1976

Current Grant Level: $16,892.

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Buccal Absorption of Nicotine in the Anesthetised Cat.

The investigators propose to perform a series of 12 experiments on anesthetised male cats to investigate the buccal absorption of ^{14}C-labeled nicotine from tobacco smoke using six cigarettes and six cigars which have been labeled with ^{14}C-nicotine. Blood levels of ^{14}C-nicotine and its metabolite, ^{14}C-cotinine, will be measured as will the distribution of these compounds in tissues such as brain, liver, lungs, kidney and gastrointestinal tract.

The results should help to explain, in pharmacokinetic terms, what differences exist between buccal absorption of nicotine from an essentially "acid" cigarette smoke and an "alkaline" cigar smoke.

Activation Date: September 1, 1976 to February 28, 1977

Current Grant Level: $18,500.

Union Carbide Corporation,
Nuclear Division
Oak Ridge, Tennessee 37830

Characterization of Animal Inhalation Exposure Devices.

The purposes of this program are to collaborate in the development of advanced tobacco smoke inhalation exposure methods and to provide chemical and physical characterization of tobacco smoke offered by exposure devices of interest to The Council for Tobacco Research - U.S.A., Inc. Carefully defined exposure devices and methods are critical to the proper design and final interpretation of biological studies requiring inhalation dosing with whole smoke. Experimental studies will emphasize (a) in-depth characterization and evaluation of the Process and Instruments Smoke Exposure Machine (SEM) II, (b) completion of ongoing studies to characterize the Walton Horizontal smoking machine, and (c) initiation of a chemical/instrumentation support activity to provide control and documentation of chronic mouse inhalation experiments.

The Process and Instruments SEM II, now in the final stages of construction, will receive a thorough operational characterization and evaluation to establish the practicality and reliability of the system for large-scale inhalation exposures. The following operational features will be investigated: humidity and temperature of puffing and purge air, consistency of dome pressure, practicality and reliability of puff-volume calibration method, system components reliability under heavy usage, operational ease, and service and cleaning requirements.

Activation Date: May 10, 1976

Current Contract Level: $290,000.

Union Carbide Corporation,
Nuclear Division
Oak Ridge, Tennessee 37830

Dosimetry Studies on the Process and Instruments Smoke Exposure Machine.

Detailed studies will be made to establish the deposition of tobacco smoke particulates in mice exposed on the Process and Instruments Smoke Exposure Machine (SEM II). The work will define the dose of smoke received under exposure conditions being considered for inhalation studies with the SEM II. A series of experiments will be performed to measure deposition as a function of smoke concentration, exposure time, and smoke flow rate through the animal containment units. Results of this study will aid in the selection of the best conditions for long-term chronic inhalation studies.

Other studies will address basic questions related to the dose of smoke particulates received by mice in inhalation studies and new methodological approaches to measure smoke uptake by animals. The possible effects of smoke particle size on the total deposition and deposition site in the respiratory tract will be investigated using the SEM II to generate smoke aerosols of abnormally large particle size. The effect of animal acclimatization on smoke deposition will be investigated as part of other long-term exposure experiments. Efforts will be made to correlate smoke deposition with the time-integral of smoke concentration offered animals, the carbon dioxide exhaled by the animals during exposures, and the carboxyhemoglobin level in the blood of animals at sacrifice. If one or more of these parameters are found to correlate well with particulate dosimetry, methodology will be developed for monitoring the parameter in long-term exposures to provide continuous, non-destructive dosimetry during inhalation exposure experiments.

Activation Date: April 15, 1976

Current Contract Level: $115,000.

Guenther Boden, M.D.
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Assistant Director, General Clinical
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Temple University Health Sciences Center
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Effect of Nicotine and Cigarette Smoke on Secretin Secretion.

The objectives of this study are: (1) to determine whether nicotine or cigarette smoke does inhibit pancreatic secretion of water and bicarbonate; and (2) if so, to determine whether the inhibition is mediated by suppression of the gastrointestinal hormone secretin or by a direct effect on the pancreas or both.

The acute effects of intravenously infused nicotine or inhaled cigarette smoke on basal as well as HCl-stimulated serum secretin concentrations and on pancreatic volume and bicarbonate secretion will be studied in dogs.

In alteration of the original experimental design which the investigator has used in the past year, he will perform these studies in alert dogs carrying chronic gastric as well as pancreatic fistulas. Experimentation with alert dogs will circumvent the major problems that have been encountered using anesthetized animals. These problems consisted primarily of severe depression of exocrine pancreatic secretion as well as suppression of secretin release.

Activation Date: January 1, 1975

Current Grant Level: $37,790.

Albert Castro, Ph.D.
Director, Hormone Laboratory and
Associate Professor of Medicine
Department of Medicine
University of Miami School of Medicine
Miami, Florida 33136

Nicotine in Blood: Detection by Radioimmunoassay..

The major objective of this project is to develop means of quantifying nicotine and its major metabolites in blood at levels achieved as a consequence of tobacco smoking. In view of the low levels of substances to be measured and the investigators' experience in detecting hormones and drugs, they believe radioimmunoassay is the procedure of choice.

The project covers development of specific antisera against nicotine, cotinine, hydroxycotinine and desmethylcotinine. As antisera become available, protocols will be developed for assaying nicotine and its metabolites using "wet" chemistry techniques. However, standard radioimmunoassay methodology can be simplified and accelerated significantly by converting to "solid-phase." Specifically, as "wet" chemistry protocols are developed and field-tested, the investigators will couple specific antisera to solid supports (e.g., silanized controlled pore glass), and develop and test solid-phase protocols in parallel. Their preliminary experience with solid-phase radioimmunoassays of thyrotropin and progesterone indicate that the time required for a given assay may be reduced seventy-fold.

Little is known of the concentrations of nicotine and its metabolites achieved in blood, other body fluids, and tissues as a consequence of smoking. The investigators believe that modern assay technology can remedy this situation, and they, therefore, propose to develop radioimmunoassays for nicotine, cotinine, hydroxycotinine and desmethylcotinine. Emphasis will be placed on simplicity and rapidity of use as well as on specificity and sensitivity. They then plan to make these assays available to all interested investigators to permit direct re-examination of the actions of nicotine and its metabolites as a function of inhalation of tobacco smoke.

Activation Date: April 1, 1974

Current Grant Level: $48,545.

Naiter M. Chopra, Ph.D.
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The Degradation of DDT, TDE, and Dieldrin in Cigarette Main-Stream and Side-Stream Smokes. Grant #579R2

DDT, TDE, and dieldrin were extensively used as pesticides on tobacco plants until very recently, and for quite some time cigarettes available on the market would carry these pesticides and their degradation products. Little work has been done so far on (1) the fate of these pesticides and their degradation products in cigarette smokes; (2) the mechanisms of the degradation of pesticides in tobacco smokes; and (3) the possible hazards these pesticides and their breakdown products may cause to the smoker. We are here working on these topics.

Current Grant Level: $29,189.

Paul S. Larson, Ph.D.
Medical College of Virginia
Health Sciences Division
Virginia Commonwealth University
MCV Station, Box 726
Richmond, Virginia 23298

Abstracting and Classifying the Literature on the Biologic Effects of Tobacco, and Following Manuscript for Supplement III to Tobacco (1961) through Printing and Preparation of Subject Index.

In 1961, the investigator, together with Dr. Herbert Silvette and Dr. H. B. Haag, authored the monograph "Tobacco: Experimental and Clinical Studies," Williams & Wilkins Co., Baltimore, 932 pp. This was followed in 1968 by "Tobacco: Supplement I," 803 pp., and in 1971 by "Tobacco: Supplement II," 563 pp. Publication of "Tobacco: Supplement III" is planned for 1975. The objective throughout has been to present the reader with "A Comprehensive Account of the World Literature."

The approach for Supplement III has been patterned after the preceding three volumes.

The manuscript will be turned over to the Williams & Wilkins Co. around July 1, 1974. The new supplement is based on approximately 4,000 journal articles collected since completion of manuscript for "Supplement II." Publication is expected by mid-1975.

Activation Date: July 1, 1974

Current Grant Level: $51,796.

Mason Research Institute
Harvard Street
Worcester, Massachusetts 01608

Mechanical and Biological Evaluation of Devices for the Monitored Exposure of Experimental Animals to Tobacco Smoke Inhalation: Smoking Machine Testing, Lung Inhalation Dosage Determination.

The program to test the Walton Horizontal Smoke Exposure Machine with regard to mixing, dosage and dilution potential, is being continued. Various gas mixtures are being used as standards, the reproducibility of puffs within individual cigarettes and between different cigarettes having been found to be too aberrant for analytical purposes. Mixing and dilution are being studied using gas chromatography for gas phase, and trapped particulate at various locations within the animal exposure chambers.

Concurrently, another group of experiments using chlorinated hydrocarbons and C^{14}-labeled dotriacontane as smoke particulate labels is directed at determining lung dosage of an exposed animal. Recovery experiments, mixing tracer compound and lung tissue and analyzing recovery after incubation, are underway to determine the rate of metabolism of the compounds proposed as tracers. Ideally the compounds would not be metabolized and would be recovered quantitatively.

Activation Date: December 1, 1971 - May 31, 1972

Current Contract Level: $60,000.

Dr. Georg B. Neurath
Microanalytical Laboratory
Hexentwiete 32
2000 Hamburg 56
West Germany

Kinetics of the Nitrosamine Formation in Tobacco Smoke.

The kinetics of the formation of nitrosamines by interaction of the nitrogen oxides and their reaction with amines are being studied by means of modern physicochemical methods (chemiluminescence). The influence of several main factors on the reactions: oxygen supply, temperature, condensation, solvents, and aqueous systems with different pH-values, are under investigation.

Reactions are followed by measurement of nitrogen oxide and nitrosamine concentrations by utilization of the chemiluminescence method and gas chromatography respectively.

Normal German blend cigarettes have been shown to deliver smoke with NO contents of 540 ng/ml smoke mean value. The minimum value was found with a flue-cured tobacco filter cigarette: 180 ng/ml. Maximum value of a Spanish black filter cigarette: 1520 ng NO/ml smoke.

The half life time of NO at 500 vpm in presence of 10% O_2 was determined to be 15 minutes.

NO_2 originally does not occur in the smoke of most cigarettes.

Adaptation of the chemiluminescence method to direct determination of nitrosamines in cigarette smoke, when present, has been undertaken.

Activation Date: January 1, 1975

Current Grant Level: $46,120.

Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

Devices for the Exposure of Experimental Animals to Whole Tobacco Smoke: Chemical, Physical and Operational Characterization.

The primary experimental activity and initial goal of this contract involves the chemical, physical and operational characterization of three types of devices, supplied by the contractor, for the generation and subsequent exposure of animals to fresh cigarette smoke. The broader goal is to identify those characteristics of inhalation bioassay exposure methodologies which are critical to the development of a meaningful bioassay. Methods presently in existence will be applied to these studies while research progresses to develop the needed, more sophisticated, techniques.

The major area of this work concerns the chemical and physical definition of the smoke aerosol produced and offered to the animals. Specific questions to be addressed include the quantity and composition of the smoke offered the animals as functions of chamber position and time after introducing the puff. The influence of the animals on smoke quantity and composition through the course of an exposure must also be established. Integrated biological measures of the effectiveness of the device under study must be determined by lung dosimetry studies. The preferred animal containment system, not yet identified for these devices, will be identified by way of an extensive study of respiratory patterns of exposed animals. These and other miscellaneous studies will define the behavior of the smoking/exposure devices, identify needs for improvement, and establish a baseline to which other smoking/exposure devices can be related.

Activation Date: May 10, 1974

Current Contract Level: $223,500.

B. V. Rama Sastry, D.Sc., Ph.D.
Professor of Pharmacology
Vanderbilt University School of Medicine
Nashville, Tennessee 37232

Influence of Nicotine on the Release of Acetylcholine in the Human Placenta and its Implications on the Fetal Growth.

Recent surveys indicate that babies of women who are habituated to smoke tobacco are smaller in size than those of nonsmokers. Further recent studies indicate that nicotine releases acetylcholine (ACh) from presynaptic nerve terminals and synaptosomes. In view of these observations, one could anticipate that nicotine can release more than the normal quanta of ACh from its stores in the villous epithelium. Released ACh (and/or nicotine) may influence the transport of energy metabolites across the trophoblast. The final result would be fetal deprivation and relatively poor fetal growth. At the present time, it is not possible to assess whether the final effect is due to released ACh or nicotine or both.

The aims of this project are: (1) to study the effects of nicotine on the release of ACh from human placental villi and to establish relationships between the dose of nicotine and ACh released into the medium (or ACh retained in villi); (2) to separate the ACh granules from placental tissue by density gradient separation, and to study the mechanisms of ACh release from granules by nicotine; (3) to obtain evidence for the presence of a cholinergic receptor in the human trophoblast where nicotine binds for exhibiting agonistic or antagonistic effects; and (4) to set up a perfusion system for human placenta for use in the investigators' laboratories.

The existence of an ACh-choline acetylase-acetylcholinesterase-like system in human placenta was demonstrated in the researchers' laboratories. In view of this, the above specific aims will be pursued in the immediate future.

Activation Date: May 1, 1974

Current Grant Level: $12,806.

John R. Rowlands, Ph.D.
Department of Physical and Biological Sciences
Southwest Research Institute
8500 Culebra Road
San Antonio, Texas 78228

Spectroscopic Investigation of Tobacco Smoke Constituents on Mammals.

This experimental plan, in essence, will consist of isolating mammalian cells and cell membranes, exposing them in vitro to cigarette smoke components, labeling them with nitroxide spin labels, dialyzing to remove any unbound labeling compound, and obtaining electron spin resonance measurements of the labeled cells and membranes.

The hemoglobin obtained upon hemolysis of the red blood cells (control and exposed) will be examined by magnetic circular dichroism (MCD), optical, and ESR spectroscopy in order to detect differences in the heme moiety.

Activation Date: October 1, 1970

Current Contract Level: $51,610.

Barbara B. Brown, Ph.D.
Veterans Administration Hospital
1611 Plummer Street
Sepulveda, California 91343

Exploration of the Differences in EEG Pattern - Subjective State Correlates in Smoker and Non-smoker Subjects.

Analysis of data accumulated in the past two years strongly suggests that central information processing not only differs radically between cigarette smokers and non-smokers, but that these two populations differ in their modes of both active perception and recall, and suggests further that these tendencies are genetic in origin.

In this year's work, physiologic recording is being expanded to include EEG activity from bitemporal and precentral electrode placements as well as from conventional bilateral arrays, and to record both horizontal and lateral eye movements. The EEG additions are for study of differential activity between alpha and kappa rhythms and study of wave propagation times to document differences in central information processing activity. Recording as much eye movement activity as possible has been found helpful in documenting preferred modes of perception and of recall. Documenting the EEG and eye movement indicators of these mental functions assists in further differentiation between the subject groups. Additional characteristics of mental function will be documented by recording the extra scalp sites and eye movements during performance of various types of mental activity (visual, auditory and tactile perception and recall, both with eyes open and with eyes closed, and during mental tasks and cognitive functioning).

Although current information concerning the smoking habit and the CNS mechanisms involved appear quite chaotic, our past study of EEG patterns has resulted in a fairly solid foundation for these continuing studies.

Activation Date: January 1, 1971

Current Grant Level: $23,086.

Charles G. Cochrane, M.D.
Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

The Mediation of Inflammatory Injury of Tissue.

The objectives of this project are:

(a) To study the mechanism of activation of the kinin forming, intrinsic clotting, and fibrinolytic systems.

(b) To study inflammatory injury mediated by leukocytes and other cells.

The investigators plan to employ purified components of the Hageman factor activated systems which can be trace labeled with radioisotopes, and to use specific antibodies directed to these components in order to determine the participation of these systems in disease states. Leukocytes will be transferred to animals prepared with antigen and antibody, but depleted of their own neutrophils, in order to reestablish leukocytes and dependent injury. A variety of agents known to inhibit the leukocyte functions will then be interposed to control the developing injury.

The investigator has gained experience in purifying and labeling components of the Hageman factor activated system and has prepared antibodies to them; he is thus in a position to assay their participation in diseases of rabbits and man. In addition, the ability to transfer leukocytes successfully and reestablish inflammatory injury has recently been gained in this laboratory. The feasibility of these important experiments is now apparent.

Activation Date: July 1, 1974

Current Grant Level: $16,560.

Edward F. Domino, M.D.
Department of Pharmacology
University of Michigan
Ann Arbor, Michigan 49104

The Effects of Nicotine and Tobacco Smoking on the Central Nervous System.

The guiding hypothesis of this research is that people smoke for the neuropsychopharmacological effects of nicotine. The actions of nicotine and tobacco smoke are being investigated on the central nervous system of both animals and man using standard neuropharmacological tests. The following sites and mechanisms of action of nicotine are under investigation:

1. As a skeletal muscle relaxant.
2. As an agent that stimulates the brainstem activating system, producing a wakeup effect and an increase in REM sleep.
3. As an excitatory modulator of lateral geniculate transmission.

Activation Date: January 1, 1971

Current Grant Level: $32,739.

Walter B. Essman, Ph.D., M.D.
Professor of Psychology and Biochemistry
Queens College of the City University of New York
65-30 Kissena Boulevard
Flushing, New York 11367

Metabolic Response to Stress - Tobacco Smoke Interactions.

This project is directed toward the evaluation of the nature and sequence of metabolic and biochemical changes resulting from stress and/or its systems showing adaptation to stress, and their interaction with the effects produced by nicotine. The basis of the objectives of this work is a definition of those cellular and subcellular units, the biochemical or metabolic composition of which may be altered by either stress, nicotine treatment or the interaction of the two. The indices of stress and the interactive effects thereupon contributed by nicotine treatment will reside within a consideration of biologically active molecules which characterize a cellular or organelle system with which they are classically identified. As such, changes in the content, storage pool levels or turnover of these molecules may well provide extremely sensitive indices of how stress and nicotine treatment interact.

It is not the purpose of this research to explore the multifaceted systems within which stress operates or nicotine treatment exerts measurable effects, but to focus primarily upon five tissue systems from which the cellular and subcellular biochemical data may be derived in terms of those molecules which appear to be most significant for the regulation of physiologic and pathophysiologic processes within such organ systems. Those hypotheses which are generated from the investigator's research findings as well as other related work are: (1) models of acute and chronic stress, respectively, may be developed and may reflect changes that are coincident with the onset, duration and adaptation to stress; (2) several of those changes which are utilized as indices of stress onset, permanence or reversibility may be interacted upon by the effects of nicotine; (3) the nature of the biochemical or metabolic changes observed within representative cellular model systems; and (4) the objective role of nicotine, which serves to either alter the time course for onset of stress indices or change the sequence over which they may occur or be modified will be assessed.

Progress has been significantly made in several areas, notably between stress conditions and the treatment with nicotine and/or tobacco smoke in experimental animals. These findings essentially have implicated nicotine to both the regional and cellular bases in the central nervous system in its interaction with the environmental and physiologic sources of central nervous system stress. Subcellular localization of such effects and the localization of specific synthetic processes with which such interaction is interrelated have been specified.

Activation Date: January 1, 1975

Current Grant Level: $61,254.

Leonide Goldstein, D.Sc.
Associate Professor of Psychiatry
Institute for Mental Health Sciences
College of Medicine & Dentistry of New Jersey
Rutgers Medical School
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The "Chronic Nicotine State" and Anxiety: A Behavioral and Electroencephalographic Analysis of Induced and Spontaneous Hyper-activation in Rats.

The current project represents a continuation of a line of research. The investigators' electrophysiological and behavioral evidence to date indicates that chronic nicotine treatment produces shifts in the balance between reticular formation (RF) and limbic influences on arousal, resulting in a state of enhanced "motivational (limbic) arousal" and reduced "drive (reticular) arousal." Recent data suggest that nicotine can protect from the disruption of electrically-induced RF overdrive. The present study is aimed at determining whether chronic nicotinization will ameliorate the behavioral disruption resulting from spontaneous states of RF overactivation (possibly, analogous to anxiety states).

It is possible to separate more highly emotional or anxious animals from low anxiety animals by behavioral measures. Further, it has been shown that high and low emotionality strains of rats will respond differentially to various psycho-active drugs. The investigators propose to test the relative effectiveness of nicotine's protection from stress-induced behavioral disruption in rats with constitutionally different spontaneous arousal levels and to characterize the cortical and subcortical electroencephalographic indices of their functional states.

These researchers propose to examine the consequences of chronic nicotinization and withdrawal in rats separated into low anxiety and high anxiety or emotionality categories on the basis of base-line behavioral measures. If rats classified as highly anxious are in that state because of relative RF predominance in the modulation of arousal, then chronic nicotine treatment should render them less sensitive to stress; i.e., they should be less susceptible to behavioral breakdown in anxiety-provoking situations than are highly anxious rats not treated with nicotine.

Activation Date: January 1, 1975

Current Grant Level: $38,000.

Shirley L. Kauffman, M.D.
Professor of Pathology
Downstate Medical Center
State University of New York
450 Clarkson Avenue
Brooklyn, New York 11203

Morphometric Study of MouseLung Exposed to Carcinogens.

Use of ^{3}H thymidine to label respiratory epithelium combined with thin plastic sections to give higher resolution for cell identification has made it possible to outline the cell cycle of bronchiolar and Type 2 alveolar epithelium and their disturbances during acute and chronic urethane exposure.

These methods combined with quantitative morphometric techniques for study of lung, which have been developed, are adaptable to an analysis of lung carcinogenesis. The objectives of the proposed study are: (1) to work out quantitative methods for analysis of progressive cellular changes during chemically induced epithelial hyperplasia and neoplasia, and (2) to study the association of periods of increased cell proliferation in the lung and susceptibility to carcinogens.

Activation Date: October 1, 1972

Current Grant Level: $25,000.

Thomas C. Westfall, Ph.D.
Associate Professor of Pharmacology
Department of Pharmacology
University of Virginia School of Medicine
Charlottesville, Virginia

Action of Nicotine on Peripheral and Central Neurones in Animals Chronically Exposed to Nicotine.

The main objective of this study is to compare the effect of nicotine on several parameters of neuronal activity when administered to naive preparations (tissues obtained from animals not previously exposed to nicotine) or tissues obtained from animals which have been constantly exposed to nicotine for varying lengths of time.

The parameters to be measured are: (1) release of norepinephrine from peripheral adrenergic neurons (perfused heart preparation); (2) release of norepinephrine, dopamine, or serotonin from central neurons (perfused brain slice preparation; (3) turnover of norepinephrine, dopamine, or serotonin; and (4) monoamine oxidase activity and catechol-O-methyl transferase activity. The study is based on the fact that we have very reliable and reproducible methods for measuring these effects and that smokers are constant users of tobacco.

By comparing the effect of nicotine on tissues obtained from animals which have not been previously exposed to nicotine with those that have been exposed for varying periods of time, we hope to have a more valid means of correlating the effect of nicotine on the nervous system in smokers and nonsmokers.

Activation Date: January 1, 1974

Current Grant Level: $21,953.

Herbert McKennis Jr., Ph.D.
Professor of Pharmacology
Medical College of Virginia
Health Sciences Division
Virginia Commonwealth University
Richmond, Virginia 23219

Biological Activity of Tobacco Smoke and Allied Substances.

The aim of this project is to obtain information on the role of nicotine metabolites and other substances in producing or altering biological effects ordinarily ascribed to nicotine.

An example of the experimental approach comprises the isolation and separation of the quaternary ammonium compounds produced in the metabolism of nicotine through concentration on cationic exchange resins. Crude and purified fractions are subjected to bioassay on the surgically prepared forelimb of the dog. Changes in vascular resistance are determined after administration of the metabolites.

Nicotine isomethonium ion under the experimental conditions produced an increase in peripheral vascular resistance. Termination of the physiological response appears related to a metabolism of the isomethonium ion to cotinine methonium ion, while initiation of the response may be related to an unidentified metabolite. Factors related to these and related phenomena are under study.

Activation Date: October 1, 1972

Current Grant Level: $60,000.

Nicotine Effects on Behavior

1005075582

Carlton K. Erickson, Ph.D.
School of Pharmacy
University of Kansas
Lawrence, Kansas 66044

Effects of Nicotine on Free Acetylcholine in the Hippocampus During Learning.

A number of investigators have shown that nicotine, in small doses, facilitates learning in rats. Previous work in this laboratory has strongly suggested that nicotine-mediated learning facilitation is the result of a direct enhancement of the consolidating memory trace.

The current objectives are:

(a) To determine the effects of nicotine on the release of free acetylcholine (a probably neurotransmitter in the brain) from the hippocampus (a brain area possibly involved in learning) of restrained but awake rabbits.

(b) To perform similar experiments in rats that are actively learning a conditioned avoidance response.

The following approach will be used:

(a) Push-pull cannulae will be inserted into the hippocampus (unilaterally) of rabbits and rats and the brain perfused with Locke's salt solution.

(b) Analysis of levels of free (released) acetylcholine will be performed with a bioassay method, the dorsal longitudinal muscle of the leech, which is very sensitive to small amounts of acetylcholine.

(c) In rats, the technique will be developed so that acetylcholine can be measured during actual learning of an avoidance response.

(d) Nicotine will be given to determine how it affects the release of acetylcholine and how these changes correlate with speed of learning.

Plans are to correlate results obtained in this project with earlier nicotine-learning work in this laboratory.

Activation Date: June 1, 1972

Current Grant Level: $10,550.

Walter B. Essman, Ph.D.
Queens College
City University of New York
Flushing, New York 11367

Studies of Nicotine Action upon Memory Consolidation. Grant #623-A

The purpose of the research project is to assess the possible role of nicotine in determining the temporal character of memory consolidation in experimental animals. These experiments represent an attempt to relate the effects of nicotine in the central nervous system to behavioral processes dependent upon such central events. Specifically, the effect of nicotine upon brain amine concentration and turnover will be related to the process of memory consolidation (the process by which the memory trace becomes fixated in the central nervous system; this may be empirically defined in terms of the time interval following learning within which memory for an acquired event may be disrupted by externally presented agents and/or events.) Since previous work in this laboratory has shown that the temporal course of memory consolidation may be lengthened or shortened, depending upon changes in brain amine levels, specifically serotonin, the present studies are based upon the assumption that nicotine-induced changes in brain amine levels will serve as a basis for faciliatated memory consolidation. The parameters to be considered are nicotine dosage and the intensity of the amnesic event, which for present experiments will be electroconvulsive shock. A one-trial conditioning technique will be utilized to establish a passive avoidance response, the retention of which will be tested, based upon the interaction of the effects of nicotine and electro-convulsive shock upon the central nervous system and upon memory consolidation as defined by the behavioral method employed.

Current Grant Level: $17,445.

S. N. Pradhan, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
520 W Street, N.W.
Washington, D. C. 20001

Effects of Nicotine on Behavior and Its Interaction with Drugs.

Grant #548-A

Effects of nicotine have been investigated on various schedules of behavior (e.g., shock avoidance; fixed-ratio; fixed-interval, variable-interval and variable-ratio reinforcement; differential reinforcement at low rates; self-stimulation, self-administration, etc.) in experimental animals in this as well as many other laboratories. Aims of this project are:

(i) To investigate the effects of nicotine on some of these schedules in further details in order to understand the nature of its action.

(ii) To extend the studies to other schedules of behavior.

(iii) To study its interaction with various drugs (e.g., autonomic, neurotropic or otherwise) in order to modify the induced behavioral changes and to investigate the mechanism of its action.

Current Grant Level: $11,490.

Nicotine Effects on Brain & N.S.

Barbara B. Brown, Ph.D.
Experimental Psychiatry
Veterans Administration Hospital
16111 Plummer Street
Sepulveda, California 91343

Exploration of the Differences in EEG Pattern - Subjective State Correlates in Smoker and Non-Smoker Subjects. Grant #381-AR1

The proposed study will consist of three major divisions:

Part A. To determine whether the characteristic EEG pattern of heavy smokers (Brown, Neuropsychologia, 1968) is a constitutional characteristic by studying the EEGS of former heavy smokers, one group of which stopped smoking ten years ago and one group of which stopped smoking five years ago.

Part B. The computer analysis of EEG profiles of individuals who are subject to coronary attacks and compared to comparable controls, the subjects being classified as A or B types by the techniques of Dr. Rosenman and Dr. Friedman (Mount Zion, San Francisco).

Part C. Further characterization of heavy, former, heavy, average and non-smoker subjects with respect to subjective activities and their EEG and autonomic correlates. Emphasis is being placed on this part of the study in this year, a renewal year, of the study.

Current Grant Level: $18,700.

Edward F. Domino, M.D., M.S.
Department of Pharmacology
University of Michigan
Ann Arbor, Michigan 49104

The Effects of Nicotine and Tobacco Smoking on the Central Nervous System.

Grant #631R2

The guiding hypothesis of this research is that people smoke for the neuropsychopharmacological effects of nicotine. The actions of nicotine and tobacco smoke are being investigated on the central nervous system of both animals and man using standard neuropharmacological tests. The following sites and mechanisms of action of nicotine are under investigation:

1. As a skeletal muscle relaxant.

2. As an agent that stimulates the brainstem activating system, producing a wakeup effect and an increase in REM sleep.

3. As an excitatory modulator of lateral geniculate transmission.

Current Grant Level: $30,821.

Leonide Goldstein, D.Sc.
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Institute for Mental Health Sciences
College of Medicine & Dentistry of New Jersey
Rutgers Medical School
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Behavioral and Electrophysiological Effects of the "Chronic Nicotine State" in Rats.

Electrophysiological studies in this laboratory have suggested that chronic nicotine treatment produces changes in the cerebral mechanisms involved in the production and maintenance of cortical activation, namely a shift toward predominance of hippocampal influences and reduction in reticular influences on the mediation of activation. Our behavioral studies have indicated that this shift is accompanied by a qualitative change in the nature of arousal: specifically, from more general "drive-oriented" to "goal-oriented" arousal. Major experimental objectives involve the further characterization of central nervous system arousal mechanisms and the behavioral concomitants of specific levels of arousal. Such information should lead to a better understanding of the motivations which account for the widespread self-administration of nicotine by humans.

A visual attention task (which has been shown to be particularly sensitive to modifications in the state of arousal) is used to assess the effects of the chronic nicotine state on the behavior of rats. The brain electrical activity (EEG) is monitored from the cortex, hippocampus, and reticular formation of rats which have had chronic electrodes placed in those structures. The EEG data are analyzed by quantitative amplitude integration methods and the inter- and intra-structural changes occurring as a result of chronic nicotinization and/or other electrophysiological and pharmacological manipulations are correlated with behavioral changes.

Our recent electrophysiological and behavioral evidence supports the notion that the shifts in the balance between reticular and hippocampal influences produced by chronic nicotinization have an effect on the functional nature of arousal and thus, on ability to acquire and perform certain behaviors. We are continuing our investigations of the functional consequences of states of arousal with studies which incorporate pharmacological and direct electrophysiological (via brain electrical stimulation) and manipulations of arousal. Currently, the modifications of brain electrical and behavioral responses to psycho-active substances caused by the imposition of a "chronic nicotine state" on experimental rats is being studied.

Activation Date: January 1, 1974

Current Grant Level: $33,350.

John A. Rosecrans, Ph.D.
Associate Professor of Pharmacology
Medical College of Virginia
Virginia Commonwealth University
Richmond, Virginia 23219

State Dependent Properties of Nicotine Related Compounds.

The behavioral effects of various analogs and metabolites of nicotine will be studied in relation to: (1) the ability of such compounds to produce behavioral effects similar to those of nicotine, and (2) the ability of these compounds to block nicotine's behavioral effect.

The rationale of this study is based upon the ability of nicotine to act as a discriminative stimulus to ongoing behavior. In the paradigm, rats are trained to press one lever for food when under the nicotine state and the opposite lever when in the non-drug state. Once rats are trained to discriminate between drug states, then one can determine whether selected compounds are perceived by the rat as like nicotine or the non-drug state. Using this procedure, one can also detect whether a given compound can act as an antagonist to this behavioral effect of nicotine.

Five groups of rats have been trained to discriminate either different doses of nicotine (100-400 µg/kg) or are being trained at one dose (400 µg/kg), but on different schedules. Dose transfer studies and reference drug (amphetamine) transfer studies have been conducted. Intraventricular cannulas have also been implanted and generalization studies involving specific compounds are now underway.

Activation Date: January 1, 1975

Current Grant Level: $13,975.

Ulrich Schaeppi, M.D.
Mason Research Institute
21 Harvard Street
Worcester, Massachusetts 01608

Changes in EEG and Behavior Induced with the Protracted Intravenous Administration of Small Doses of Nicotine in Unrestrained Cats. Grant #712R1

In cats the i.v. infusion of nicotine in doses of from 10 to 50 µg/kg produces behavioral arousal with movements and hyperpnea. These changes are usually followed by immobility and later by sedation or sleep. Changes in the electrocorticogram include initial desynchronization or flattening of the EEG followed by EEG hypersynchrony.

In the proposed investigation cats are prepared for chronic EEG recordings and for chronic i.v. infusions. Nicotine is infused to cats performing an operant food reward response with fixed intervals. Nicotine-induced changes in the EEG from cortex, thalamus, hippocampus and amygdala are compared with the effects of arousing physiological stimuli.

Current Grant Level: $17,140.

Charles G. Cochrane, M.D.
Scripps Clinic and Research Foundation
476 Prospect Street
La Jolla, California 92037

The Mediation of Inflammatory Injury of Tissue.

The objectives of this project are:

(1) To examine the participation of components of the Hageman factor pathways in the development of inflammatory injury of the lung.

(2) To determine the role of complement and cellular factors in inflammatory pulmonary disease.

The participation of plasma proteins of the Hageman factor and complement pathways and of inflammatory cells will be examined by use of radiolabeled protein components and labeled cells. Accumulation of these components in the lung will be followed and the effect of deprivation of each determined.

Methods of isolating and radiolabeling of components have been worked out. Models of pulmonary inflammation are now being studied.

Activation Date: July 1, 1976

Current Grant Level: $31,769.

Norman W. Heimstra, Ph.D.
Professor of Psychology
Director, Human Factors Laboratory
University of South Dakota
Vermillion, South Dakota 57069

Effects of Smoking Deprivation on Group Problem Solving Processes.

Increasingly, smokers are encountering situations where they are not allowed to smoke because of restrictions that have been imposed. Previous research has shown that smoking deprivation has an effect on various psychomotor tasks with decrements in performance typically shown. The current study is concerned with determining whether smoking deprivation affects group performance, both in terms of accuracy and speed on group performance tasks and in terms of social interactions shown by members of the deprived groups in comparison to nondeprived groups.

Triads consisting of smokers who are allowed to smoke, nonsmokers, and smokers who are not permitted to smoke, will perform group problem solving tasks for several hours. Dependent variables will consist of speed and accuracy measures. Groups will also be observed and recordings of behavior obtained, in order to determine if social processes differ between groups.

Data have been collected for one year on this project.

Activation Date: October 1, 1974

Current Grant Level: $13,141.

Norman W. Heimstra, Ph.D.
Professor of Psychology
Director, Human Factors Laboratory
University of South Dakota
Vermillion, South Dakota 57069

Effects of Smoking Deprivation on Risk-Taking Behavior.

Smoking has been shown to modify various affective states of individuals. It is likely that certain aspects of behavior may be associated with these changes in affective state and that smoking deprivation may thus influence these kinds of behavior. It would seem that risk-taking behavior would be particularly susceptible to modification by the stress and changes in affective states brought about by smoking deprivation. This investigation is designed to determine whether this is the case.

Subjects for the study will be male college students who, on the basis of questionnaire data, have been identified as smokers. They will be assigned to two groups - one where all subjects are deprived of smoking for a number of hours prior to testing and a second group where subjects are allowed to smoke prior to and during testing. Testing will be accomplished on two devices which have been designed to measure "risk-taking" behavior of subjects.

Study is just beginning.

Activation Date: December 1, 1974

Current Grant Level: $10,727.

OTHER STUDIES OF SMOKE EFFECTS

1005075596

Illinois Institute of Technology Research Institute
10 West 35th Street
Chicago, Illinois 60616

Bioenergetic and Endocrine Studies on Cigarette Smoke and Stress Interrelationships.

The research program is designed to extend our knowledge with regard to the earlier finding that gaseous components of cigarette smoke have different effects upon electron transport and phosphorylation coupling at different cytochrome loci in mitochondria. Hence in the research program proposed for the following year, the emphasis will be placed upon the inhibitory effects of smoke components upon cytochrome oxidase and consequent interactions in the electron transport chain upon coupling activity at cytochrome b. Work toward the identification of the site II uncoupling factor and study of its mechanisms of release from the catecholamine-stimulated cell plasma membrane, as well as the mitochondrial uncoupling, will continue. Attempts will be made to develop a stress assay system for human application using several types of accessible cells.

Activation Date: June 1, 1972

Current Contract Level: $111,847.

Willis H. Riesen, Ph.D.
IIT Research Institute
10 West 35th Street
Chicago, Illinois 60016

Effects of Tobacco Smoke on Cellular Respiration. Grant #620R2

The objective is to investigate the effects of cigarette smoke inhalation upon cellular oxidative respiration in the lung. Guinea pigs are exposed to the Walton smoking machine under acute conditions of smoke exposure, and under chronic conditions that more nearly simulate human smoking parameters. While exposed, animals are confined with their noses plugged to induce smoke inhalation by mouth. The acute smoke exposure regimen produces a slight reduction of oxidative phosphorylation efficiency in lung mitochondria tested with either NAD-linked or FAD-linked substrate. However, animals that are sham exposed to the machine under conditions of confinement and nose closure uniformly show a reduction in phosphorylating efficiency by one P/O unit. Most of this reduction is reversed by inhaling smoke.

These preliminary results suggested that the stress of sham exposure may be partially alleviated in the animals exposed to smoke under acute conditions. In further studies we have identified the patterns of phosphorylative coupling at several different sites in the respiratory chain. The phosphorylation pattern of stressed animals differs from that of smoking animals in terms of site specificity. Future studies will concentrate on more prolonged chronic exposures and will include some histochemical and morphological studies.

Current Grant Level: $47,292.

Simon H. Wender, Ph.D.
University of Oklahoma
Norman, Oklahoma 73069

Effect of Cigarette Smoke and Its Components on Free Proline in Animal Tissue.
Grant #643R1

Proposed and in-progress research includes:

(1) Studies on the effect in animal tissue cultures of certain known carcinogenic agents on proline metabolism.

(2) Fractionation of cigarette smoke and subsequent study of some of these fractions on proline.

(3) Studies attempting to learn the biochemistry involved in those cases above where proline concentration is affected.

Current Grant Level: $38,213.

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VIRGIN VINYL
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